

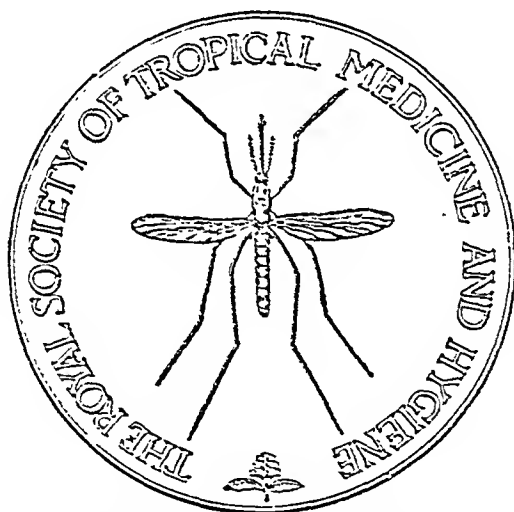


# TRANSACTIONS

OF THE

## ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

PATRON - HIS MAJESTY THE KING.



ZONAE TORRIDAE TUTAMEN

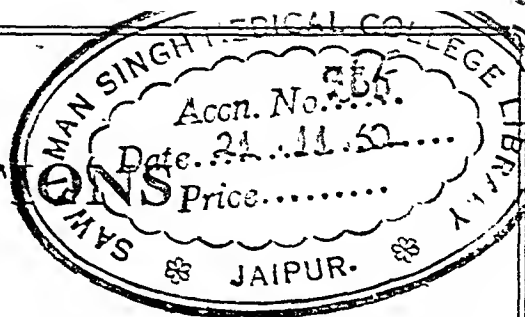
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TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL. XXXV. No. 1. JULY, 1941.

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COMMUNICATIONS.\*

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TYPHOID FEVER.

WITH SPECIAL REFERENCE TO THE VALUE OF NEW ANTISERA IN  
THERAPY AND EOSINOPENIA IN DIAGNOSIS.

BY

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PROLOGUE.

For many years typhoid fever has been endemic in Singapore and indeed throughout Malaya—the number of cases occurring in any year would in England be looked upon as indicating an epidemic—but, granted that the disease is endemic in Singapore, occasionally the numbers of cases are greatly increased and one may then justifiably speak of a local epidemic.

† I have pleasure in acknowledging the help of Dr. A. FELIX, of the Lister Institute, London, on the subject of the new antiserum. I am indebted to the Director of Medical Services, Straits Settlements, for permission to publish.

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\* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

During the months of May to August, 1938, there were admitted to the General Hospital, Singapore, 463 cases diagnosed as typhoid fever. The total number of cases in the town was no doubt far more than this, for though the majority of cases diagnosed by private practitioners as typhoid were sent to this hospital, a number of cases may easily have escaped diagnosis—and indeed large numbers of sick people in Singapore do not call in a qualified practitioner and may go through life without ever having consulted a doctor.

The number mentioned—463 cases in 4 months—may be compared with 625 cases in the General Hospital diagnosed as typhoid fever for the whole of 1938; and the 625 in turn may be compared with 185 for the whole of 1937 and 343 for the whole of 1936.

It is the purpose of this paper to analyse these 463 cases admitted in May to August, 1938, and to deduce from this analysis, if possible, criteria that may be of help in the future diagnosis and treatment of the disease. It should at once be stated that the term “typhoid fever” in this paper means invasion of the body by *Bacillus typhosus* of Eberth and that paratyphoid fever, which is incidentally quite rare in Singapore, is not included.

#### ORIGIN OF THE EPIDEMIC.

The beginning of the epidemic in May occurred amongst a fairly well-to-do portion of the community and their children; I noticed during the first week in May that the number of cases amongst this class in the hospital was increasing, the usual incidence in Singapore being high in the coolie class but not amongst more prosperous people; the Municipal Health authorities were accordingly forthwith informed of this fact, which I felt would not be noticeable in the routine return of infectious diseases because the total number of notifications from the hospital was not obviously increasing at the same time.

In the Annual Report of 1938 by the Municipal Health Officer, Singapore, on page D 3, he describes how on May 31st he was informed by the headmaster of a certain school that four of his pupils were away with typhoid fever. This group of schoolboys attacked by the disease were found to have eaten ice-cream bought from the same hawker. From this further clue and others the epidemic was traced to food and “carriers,” especially ice-cream hawkers and a particular native ice-cream factory.

#### DIAGNOSIS.

Of the 463 cases, I will first cut out 37 from the analysis as the diagnosis in these, although made on reasonable grounds, was not absolutely conclusive; the remaining 426 were proved by the occurrence of a long febrile illness associated with one or more of the following signs:—

(a) A high or rising titre of agglutination of the typhoid bacillus (Widal reaction) by the blood serum of the patient; no reading below "O" 1:100 was accepted as diagnostic.

(b) The culture of *Bacillus typhosus* from the blood or urine or stools or pus from an abscess or other body fluid.

(c) The occurrence of gross intestinal haemorrhage.

(d) The occurrence of perforation of an ulcer of the ileum.

(e) The postmortem finding of typhoid ulceration of the bowel.

#### RACIAL COMPARISON AND EFFECT OF INOCULATION.

There were only two cases of Europeans in the group; both recovered and it is fairly certain that these were the only European cases in the town. There was at least one European to every fifty others in Singapore, a town with a population of half a million odd, so that the incidence in Europeans was definitely low. Many of the European population had been inoculated against typhoid whereas very few of the rest of the population had been so inoculated; during the epidemic however large numbers of the populace were inoculated, especially school children and employees of large firms and this no doubt contributed to a fairly rapid control of what threatened to be a very severe outbreak.

#### SIGNS, SYMPTOMS AND COMPLICATIONS.

The illness in general was mostly of a severe type and of the classical textbook variety; prolonged fever and malaise, prostration, low muttering delirium, bronchitis, subsultus tendinum, cardiovascular dysfunction, and so on, were so common as to be usual features. Peripheral neuritis was not uncommon; to some extent this may have been due to latent beriberi brought on by the disease, but it tended to affect the diaphragm which is unusual in beriberi; the neuritis tended to disappear quickly if the patient recovered, much more quickly than is usual in nerve beriberi; the injection of 10,000 or 15,000 units of vitamin B<sub>1</sub> seemed definitely helpful in several of the neuritis cases, as has also been found in cases of neuritis due to various other causes.

There was clinical evidence of encephalitis in many of the cases, but the cerebrospinal fluid in these was usually normal, and only occasionally showed evidence of meningitis with presence of many cells, chiefly lymphocytes; the typhoid bacillus was not successfully cultured from the cerebrospinal fluid of any of the cases between May and August, 1938, but we have had a positive culture in a case of typhoid meningitis since then. Parotitis (sometimes suppurative), otitis media, and pneumonia were fairly common complications; epistaxis, bleeding from the gums, purpuric rashes, phlebitis, and abscesses were rarer; no case of typhoid osteomyelitis occurred.



*Mortality Analysis.*

It may be as well to precede this by the following table showing the percentage case fatality rate from typhoid fever in the years 1933 to 1938 ; it must be remembered that cases are usually sent to this hospital when the disease has already become serious.

Year	Percentage of deaths from typhoid to total treated as typhoid.
1933 ...	27·3
1934 ...	31·7
1935 ...	26·6
1936 ...	25·1
1937 ...	18·7
1938 ...	23·3

Ninety-four deaths occurred in the series of 426 cases analysed in this paper, *i.e.*, 22 per cent., and a brief description of the immediate causes of death may be of interest ; in sixty, toxæmia alone was given as the cause ; in four, toxæmia with hyperpyrexia ; in one, perforation and hyperpyrexia ; perforation in three others ; intestinal hæmorrhage in seventeen, mostly associated with severe toxæmia and two also with pneumonia ; septicæmia, peripheral neuritis affecting the diaphragm, pulmonary oedema, pneumonia alone (three), and meningitis are given as the causes of the other deaths. The one death in the group of cases treated by the " Felix " antiserum (see later) was from toxæmia and pneumonia.

## TREATMENT.

(1) *General.*

All cases were nursed at absolute rest in bed during the febrile period and for at least one week afterwards ; by absolute rest was meant that the patient was not allowed out of bed for any purpose whatsoever.

The diet at first in nearly all cases was varied nourishing fluids, including milk and milk variants such as ovaltine, Horlick's malted milk, cocoa ; fruit juice and water, etc. ; soon semi-solids were added such as jellies, custard, blancmange, cornflour, milk puddings ; ice-cream, chocolate and plain sweets were allowed to all who could be depended upon not to swallow lumps ; as improvement occurred mashed potatoes, pounded fish and other readily digestible solids were gradually added till the diet approximated to that of a healthy person.

Frequent sponging, usually 4-hourly, was used almost as routine while fever remained high. Sulphonamide derivatives were tried in a few cases but with no conspicuous benefit, and we did not persevere with them as most cases

had a leucopenia which seemed to me a relative contra-indication for this group of drugs; having personally seen two cases (not in typhoid) of agranulocytosis due to these drugs I have been very hesitant to use them in the presence of leucopenia. HARRIES *et al.* (1939) have recently reported favourably on the use of sulphanilamide and M. & B. 693 in typhoid fever, especially in combination with the use of Felix antiserum, but there seems little confirmation.

## (2) Serum.

During May and June some anti-typhoid serum prepared by the South African Institute for Medical Research, under the guidance of the pioneer Dr. E. GRASSET, was available; this had been prepared before the importance of the "Vi" antigen was recognized; it was given subcutaneously, intramuscularly or intravenously. It had been used by us sporadically in Malaya for treating typhoid fever for some years previously and we had formed the impression that it helped certain cases. Dr. GRASSET had, so I am informed, taken Malayan cultures of typhoid bacilli as well as others in preparing his antiserum in South Africa.

The criteria taken for its use in this epidemic were as follows:—

- (1) The case should have been proved to be typhoid.
- (2) The case should be acutely toxic.
- (3) The case should be reasonably early, in the first 2 weeks of the illness if possible.

Forty-three cases were treated with this serum and there were twelve deaths among these; the average amount of serum used per case was 31 c.c. Eighteen of the cases were over 13 years of age, the other twenty-five were 13 or under. The forty-three cases were classified after the disease was over as "very severe" (25), "severe" (11), "moderate to severe" (2), and "moderate" (5). This classification referring to the whole course of an individual but prolonged illness, was of course empirical; for instance, regarding pyrexia, if the fever never rose above 100° F. the case would probably be regarded as "mild," if never above 102° "moderate," if never above 104° "severe," and if running above 104° then "very severe"—toxaemia and other signs were also taken into account in this classification, but precision was obviously difficult in assessing these points. Dr. A. FELIX, of the Lister Institute, in a personal communication suggested the use of the classification "extremely severe," "very severe," and "severe," but I felt I could hardly give all the cases the adjective severe.

During July and August, anti-typhoid serum prepared by the Lister Institute of London, under the direction of Dr. FELIX, became available and was used in seventeen cases. The difference between the South African serum that we had used and the Felix serum was that the former contained chiefly "O" antibodies while the latter had been prepared to contain "O" and "Vi" antibodies.

There have been several references in recent years testifying to the value of this serum prepared at the Lister Institute in the treatment of typhoid fever—FELIX (1935), MCSWEENEY (1935 and 1937), ROBERTSON and YU (1936), COOKSON and FACET (1937), LEWIN (1938), FELIX and PETRIE (1938), COOKSON (1939), PIJPER and CROCKER (1939) and YU (1939). Its use prophylactically is suggested by FENTON and HAY (1938) in certain circumstances. GRASSET has himself now adopted the principle of the importance of the "Vi" antibody in addition to the "O" and he and LEWIN (1937) have published an article describing how the new serum is prepared in South Africa, giving a comparison of laboratory tests between it and other sera, and mentioning preliminary clinical trials. All the anti-typhoid serum prepared at the South African Institute for Medical Research is, I am informed, now prepared against strains of bacilli, rich in both "O" and "Vi" antigens. The standardization committee of the League of Nations at its meeting in Paris, in October, 1938, came to the conclusion that the most efficient anti-typhoid serum at present available is one that contains both "Vi" and "O" antibodies in as high a titre as possible. Details of the preparation of the Felix serum we used were given by FELIX and PETRIE (1938).

Of the seventeen cases treated with the Felix antiserum in this epidemic only one case died; the average amount of serum used per case of this group was 55 c.c.; seven of the cases were over 13, ten under, approximately the same proportion as with the earlier South African serum. The criteria for its use were that the case must be (1) proved typhoid, (2) very toxic, while the previous length of the illness was not taken into consideration. Of the seventeen cases, four were classified as "severe," and thirteen as "very severe," using standards similar to those used for classifying the South African serum cases.

On the face of it, these figures suggest that the Felix antiserum was a valuable adjunct to treatment, but there is an obvious possibility of fallacies, and I had best deal with these as they occurred to me.

(1) The disease might have had a lesser virulence in July and August, if it has passed its peak in June.

(2) The serum was used in certain wards only, owing to the limited amount available, and other factors might thus have influenced the results such as age and class of patient, nursing differences, etc.

(3) The average quantity of South African serum used per case was less than that of Felix serum.

(4) Seventeen is too small a number of cases to dogmatize upon.

It would be best to deal with these points *seriatim* :—

*Point (1).*—The following table shows that the fatality rate of the epidemic had not become less in July and August than it had been in May and June. It gives a percentage of deaths from typhoid as compared to admissions of typhoid cases, month by month, and though of course it does not represent a monthly percentage mortality it does give a fair idea of the prevalent severity :—

Month	Admission of typhoid cases.	Deaths.	Percentage of deaths to admissions.
May ... ..	21	4	19
June ... ..	246	47	19.1
July ... ..	114	30	26.3
August ... ..	45	13	28.8
Totals ...	426	94	22.3

One must admit that the percentage fatality in this table for June, which was the worst month of the epidemic, should actually be higher than 19.1, and that for July and August lower, because some of the cases admitted in June died in July or even August, but only three of the thirteen cases that died in August gave a date of onset earlier than July. Thus it is reasonable to say that there was no slackening of the virulence of the disease in July and August.

*Point (2).*—The Felix serum was chiefly used in certain wards, those under my immediate supervision. The following figures give the case fatality rates of the whole 426 cases, taking wards under my immediate supervision as compared with wards not under my immediate supervision :—

		Total cases.	Deaths	Fatality rate percentage.
A	Wards under my immediate supervision ... ..	91	19	21
B	Wards not under my immediate supervision ... ..	307	72	23.5
C	Children's Wards (not under my supervision) ... ..	28	3	11
	Total ... ..	426	94	22

There is no significant difference between A and B. The lower comparative mortality in the Children's Wards, in which children up to 6 years of age are nursed, is striking, especially as the general mortality from all disease in these wards is very high—45.5 per cent. for 1938; it suggests that there is a strong inborn immunity to typhoid fever, gradually wearing off as the years pass by; there were in fact no cases of typhoid at all in children under 2 during these

4 months, though occasionally a case does occur in a child under 2. Further, in several of the cases in young children the illness was remarkably mild and appeared to last less than 2 weeks. The children in these wards were under the treatment of the same lady medical officer as were the cases in the female third-class wards, where the case fatality rate was 23 per cent. (27 deaths in 117 cases) so that it does not seem likely that any difference in treatment accounted for the lesser gravity of the disease in the children's wards.

In the schoolboys' wards on the other hand, in which the ages are mostly between 7 and 12, there were seven deaths in forty-one cases, *i.e.*, 17 per cent., only a little lower than the general case fatality rate. Fourteen of the seventeen cases treated by the Felix serum were over 10, and none under 6. Again, in the first- and second-class wards there were twelve deaths in fifty cases, *i.e.*, 24 per cent. Thus the low fatality rate of the cases treated by the Felix serum has not been accounted for by the age or class of patient so treated, or by special nursing facilities.

*Point (3).*—The dosage of South African serum used was definitely less than that of the Felix serum used ; this was because it was based on a suggested dosage of 20 c.c. to be given thrice if necessary compared to a suggested dosage of 33 c.c. for the Felix serum to be given thrice. We would have tried heavier dosage of the South African serum later for the sake of comparison, but the results with the Felix serum were so much better that we decided to continue with it, and later the South African serum itself was also produced for distribution by a similar process to that of the Felix serum. Throughout the epidemic we did not have large quantities of either serum available, so that we tended to give the suggested doses twice rather than thrice. The mortality of the cases treated with the South African serum (28 per cent.) was actually higher than that of cases not treated with serum at all ; this may be readily accounted for by the fact that they were acutely toxic cases, but it throws into greater prominence the good effect of the Felix serum, which was used for still worse cases.

*Point (4).*—Seventeen is certainly too small a number of cases on which to draw definite conclusions, but I felt that with elimination of the fallacies mentioned the series would be worth recording.

#### EOSINOPENIA.

White blood cell counts and differential counts were not done as a routine in the Singapore epidemic as a great deal of extra work was thrown on the staff in many directions and the clinical laboratories of the hospital are at all times working under high pressure. Such counts were made however in forty-three of the proved cases of typhoid fever during the epidemic for various reasons, and in thirty-seven of them no eosinophils were seen. The total white blood counts quite often did not show a general leucopenia and occasionally there was

even a leucocytosis. LOWSON analysing ninety cases of typhoid fever at this hospital, noted absence of eosinophils in sixty-five, having had differential counts made in all of them. Adding his figures to mine, we find that 102 out of 133 cases of typhoid fever showed no eosinophils, *i.e.*, about 77 per cent. of the cases, a very high proportion. The usual number of white cells counted here in a differential count is 200, not enough for strict accuracy but enough for routine work. As a control, I had differential counts taken of 100 consecutive cases of other diseases at random, and thirty-one showed no eosinophils, a much lower proportion than in the typhoid cases. I further had counts made of 1,000 white blood cells in two of the cases of typhoid fever that had shown no eosinophils by the routine method; in the thousand cells, five eosinophils only were found in one case (0.5 per cent.) and none at all in the other, as follows:—

Case (1): Total white blood count :	Case (2): Total white blood count :
7,400 per c.mm. Differential count	3,900 per c.mm. Differential count
of 1,000 leucocytes :—	of 1,000 leucocytes :—
Polymorphs .. 67 per cent.	Polymorphs .. 60 per cent.
Lymphocytes 24.5 „	Lymphocytes 34 „
Large hyalines 8 „	Large hyalines 6 „
Eosinophils 0.5 „	Eosinophils .. 0

As LOWSON remarked in his paper, the incidence of helminthiasis is high in this country, and the absent or low eosinophil count is the more remarkable; many of the typhoid cases in the 1938 epidemic had helminth ova in their stools. One case in my series was specially interesting from this point of view; in the earlier stages of his illness his count was taken twice, and on these occasions the total count was approximately 7,000 white blood cells per c.mm. and no eosinophils were seen; later in the disease he developed an abscess in the gluteal muscles; his white count went up to nearly 17,000 with 5 per cent. of eosinophils in the differential.

#### *Other Observations.*

The Widal test (agglutination titre) yielded some further points of interest. It does not always change during the course of the disease, *e.g.*, one case was proved by blood culture, being positive to *B. typhosus*, while the Widal was reported "H" 1:125, "O" 1:50 on 27.7.38 and again on 2.8.38. In uninoculated persons the "H" agglutination titre may be more valuable than the "O"; for instance, in another case the highest titre obtained was "H" 1:125, "O" 1:50, while the blood culture was positive for *B. typhosus*. Another case was a good example of the Widal being completely negative in the early stage of the disease with a positive blood culture while a little later the Widal became positive ("H" 1:500, "O" 1:250) and the blood culture was then negative.

A few remarks may be added here on the question of the advisability of compulsory inoculation as a public health measure. Typhoid fever is prevalent

in Singapore and indeed in the whole of Malaya, and until the sanitary disposal of sewage and general sanitary education of the people reaches a much higher standard, the best protection would appear to be by inoculation of all the population and immigrants. Cholera and paratyphoid fever having fortunately not so far attacked this country seriously, a simple vaccine of typhoid bacilli only is required, and this can be readily prepared locally in large quantities; our usual method is by two subcutaneous injections, with an interval of a week or 10 days, of 500 million and 1,000 million dead organisms. It has been my experience both in this epidemic and previously that the individual protection so conferred, though it may wane rapidly, nevertheless usually persists to a valuable extent for many years.

There were of course for some years after the last war many articles on the value and duration of the immunity conferred by injections of typhoid vaccines. A recent article by the laboratory staff of the American Army Medical School (SILER and DUNHAM, 1939) gives interesting confirmatory evidence but advocates that, after preliminary protection by the usual method of subcutaneous inoculation, later intradermal inoculations of 0.1 c.c. (*i.e.*, 100 million organisms) every 2 to 4 years would maintain a very high immunity. I have tested this method on myself and on a volunteer colleague (Dr. B. CHEW) and can confirm their claim and consider that the method holds great promise. In both our cases the intradermal inoculation rapidly increased the Widal agglutination titre produced by previous subcutaneous inoculation, my own rising from "H" 1:680 to "H" 1:1,000 and from "O" 1:50 to "O" 1:125, while Dr. CHEW's titre rose from "H" 1:125 to "H" 1:3,400 while showing no "O" agglutination titre. Our local reactions after intradermal inoculation were definite but not incapacitating, and general reaction was quite slight, whereas after subcutaneous inoculation and re-inoculation both local and general reactions were violent and incapacitating for a time. The intradermal route as the primary method of inoculation does not appear to produce immunity, as I have confirmed in the case of another volunteer.

#### SUMMARY.

(1) The injection of serum prepared especially against strains of typhoid bacilli strong in "O" and "Vi" antigens appeared during the epidemic at Singapore to be of value in the treatment of serious cases of typhoid fever, even at a comparatively late stage of the disease. The time for the routine use of such serum in treatment has perhaps not yet come, but strong indications for its use are severe toxæmia, or failure to improve with general treatment.

(2) The absence of eosinophil cells in a differential white blood count is of value in the diagnosis though it is not an absolute sign in either a negative or positive direction.

(3) Congenital immunity against typhoid fever appears to be powerful for several years of childhood, in Malaya and presumably elsewhere also.

(4) Compulsory inoculation is advocated as a public health measure of protection against typhoid fever in countries where the disease is endemic, but not earlier than the 5th year of age.

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## A NOTE ON INFANTILE PELLAGRA.

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Since the publication of my previous articles (TROWELL 1937 and 1941) on the question of infantile pellagra, there have appeared recently various articles which carry forward the discussion a stage further, and it would appear desirable to define the points at issue in the controversy concerning the aetiology of infantile pellagra or kwashiorkor. There appears to be no real measure of agreement about what this disease should be called: there exist in the literature at least twelve different names for it, divisible into two main groups, those which associate the disease with an undefined malnutritional state: namely kwashiorkor (West Africa), Williams's disease (West Africa), Gillan's oedema (Kenya), infantile oedema (Kenya), oedema of avitaminosis (Costa Rica), Goenz's disease (Salvador), tropical infantile oedematous cachexia (Salvador); and those which associate the condition with pellagra: namely, pellagroid-beriberi (Cuba), culebrillo (Mexico), while STANNUS (1936) calls it pellagra, as does SEQUEIRA (1938), TROLLI (1938) the cheveux blancs of vitamin B deficiency, and myself infantile pellagra.

Those who consider the matter undecided should refer to it as the Proctor-Williams's disease in honour to PROCTOR (1926) who published a brief description of the disease in Kenya and WILLIAMS (CICELY WILLIAMS, 1933) who, working on the West Coast, gave the first adequate description of the disease and named

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it kwashiorkor. It is to be hoped that the confusion created by the different writers on this disease will be terminated by referring to the disease either as kwashiorkor, infantile pellagra, or the Proctor-Williams's disease.

I would now like to define the points on which CICEY WILLIAMS and I agree and disagree. In 1933 she published the first clear description of the disease, calling it a new nutritional disease of childhood associated with a maize diet. In 1935 she replied to STANNUS's suggestion that the disease was pellagra, and called the disease kwashiorkor and considered that it was due to malnutrition. In 1940, in reply to my article, she defined again the reasons which cause her to distinguish kwashiorkor from pellagra. That these distinctions are clear in her own mind cannot be doubted for photographs from Dr. D. C. WILSON of certain cases in India revealed to her that some cases were pellagra, some were kwashiorkor, and some appeared to suffer from both diseases. The same occurred in her opinion in LOWE's cases (1931) in India, some had pellagra, some had kwashiorkor and some might have been a mixture of the two. If CICEY WILLIAMS is able to distinguish kwashiorkor from pellagra it is suggestive that she considers some cases probably suffered from both diseases and it would appear to me that even in CICEY WILLIAMS's opinion the two diseases are sometimes fairly closely related. In any case the dividing line between kwashiorkor and pellagra does not appear to be as firmly drawn as in her earlier articles of 1935.

The point of agreement between us is curiously our point of division. She was the first to suggest that the disease was due to poor nutrition and in this I agree, but she has never defined any further the nature of the deficiency. I maintain that a disease can only be proved to be due to dietetic deficiency or deficiencies when the nature of the deficiencies can be indicated even if they cannot be defined chemically; and CICEY WILLIAMS has never defined or indicated the nature of the deficiency or deficiencies. CICEY WILLIAMS does not consider that this disease has any relationship to deficiency of the vitamin B complex since there is no satisfactory proof of this. With this I am in agreement, but I consider the evidence suggestive.

There is one other small point of objection which she raises to the term "infantile pellagra," and that is that the disease is seldom if ever seen in infants. The commonest age is from 6 months to 2 years, and the disease usually occurs within a few weeks of weaning. Cases may be seen up to 10 years of age or even later but are usually seen in the first few years. In the strict etymological sense that an infant is one who does not speak, not all the cases were found in infancy, but in medical language "infantile" applies to a large age-group of young children, e.g., infantile paralysis, and the *Oxford Dictionary* defines infant as a child under 7 years of age. I am unable to accept her objections to the term "infantile." The other major vitamin deficiencies have all of them distinct

adult and infantile varieties, *e.g.*, adult beriberi and infantile beriberi, adult scurvy and infantile scurvy, osteomalacia and rickets, and it would be surprising if pellagra manifested itself in exactly the same way in children and in adults.

There is a large measure of agreement between CICELY WILLIAMS and myself. We both consider the disease is due to malnutrition, she for unspecified reasons, I because of its relationship to pellagra. We both agree that the disease is not the same as classical relapsing pellagra in adults, she by proving how dissimilar it is, I by suggesting points in which it differs from adult pellagra (acuteness, age of patient, etc.) and also in its associated deficiencies of protein leading to nutritional oedema, of iron and of the extrinsic factor of Castle and occasionally the other vitamins A, B<sub>1</sub>, C and D. I would go further: although I consider this disease closely allied to pellagra I think that further elucidation of all the fractions of the B<sub>2</sub> complex may reveal that the major deficiency in infantile pellagra is neither nicotinic acid nor riboflavin, nor pyridoxine hydrochloride (B<sub>6</sub>), nor pantothenic acid (chick anti-dermatitis factor), but some undefined X or Y substance in the B<sub>2</sub> complex, and then the name of infantile pellagra will be dropped and the correct name inserted. I do however consider that the disease bears such a close relationship to pellagra that it is along these lines that the aetiology will be finally elucidated. A persistent harping on malnutrition, general, vague and undefined, and a treatment of cases with "Vitamin A, B<sub>1</sub>, nicotinic acid and all the other nutritional adjuvants we could remember" (WILLIAMS, 1940) will solve nothing and leads nowhere.

We agree that the general depigmentation of the skin and the pallor of the hair are not specific for the disease; my earlier article (1937) makes this quite clear and I regret that CICELY WILLIAMS (1940) should feel uncertain as to my attitude in this matter. I do consider that both changes are more marked in most cases of infantile pellagra than in any other disease. Indeed it has given one name to the disease, namely, *cheveux blancs* used in the Belgian Congo. In this connection it is interesting to note that although most cachectic states cause pallor of the negro skin and thinning and straightening of the hair, yet the only vitamin which has caused this experimentally in animals is an unnamed fraction of the B<sub>2</sub> complex (LUNDE).

The points on which we differ appear to be largely two, firstly that the cheilosis (angular stomatitis) which occurs in this disease is evidence of a deficiency of the B<sub>2</sub> complex. I shall return to this later, when MANSON-BAHR's contribution to cheilosis is considered, for the time being I would state that the evidence appears to me overwhelming. CICELY WILLIAMS does not regard angular stomatitis as specific of any deficiency. It is "frequently associated with malnutrition" but occurs "in overweight gentlemen who absorb everything from caviare to peaches, and it and glossitis clear up when vitamin A, B<sub>1</sub>, B<sub>2</sub>, riboflavin and nicotinic acid are given, and frequently when no special vitamins were given." The attitude of CICELY WILLIAMS to cheilosis is comparable to

her attitude to infantile pellagra; both are vague, complex, multiple deficiency states, cured by giving every known constituent of a balanced diet.

The second point of disagreement is that the skin lesions are those of pellagra. I agree with CICELY WILLIAMS that the lesions do not appear to be exactly the same in distribution or character to those of adult pellagra. They do not appear to be red or this is not observed under the dark negro skin. They do not have a sharp edge, they do not appear on the parts exposed to the sunlight. I am still puzzled by this and I am not happy about the explanations which I have tried to offer. The different character may be due to the fact that it is occurring in young children but this is not convincing. Some pictures like those in CICELY WILLIAMS's articles show pellagrous children with skin lesions comparable to those in the adult but differing from those of infantile pellagra. Other pictures of pellagra in young children, such as those by LEWIS (1926), a case seen by GOLDBERGER and MCENERY (1933), show skin lesions which I consider identical with my cases of infantile pellagra. The distribution of the dermatosis in infantile pellagra is certainly different from that seen in adults suffering from the chronic disease, who, clothed in the European manner, show areas of dermatosis on the exposed skin of the face and hands. Cases of infantile pellagra show patches on the sites of pressure and irritation, e.g., napkin area, back, knees, elbows, etc. In this connection CICELY WILLIAMS reproves me for not having referred to the work of TURNER who states (1935): "Probably because children prefer to go out of doors without hats they are more likely to have skin lesions on the face, especially the forehead, than are adults." In African children who wear neither hats nor clothes TURNER's argument would appear to indicate that the skin lesions would then occur on the sun-exposed back, shoulders and face. They certainly occur on both of the former but very scantily on the latter. TURNER continues: "As in the adult so in the child chronically ill of some intestinal disorder which keeps him in bed, he is likely to be afflicted with skin lesions quite insignificant in size and severity. In such patients the *skin areas involved are not those commonly sun-burned* as in the case of the usual pellagrin who is out of doors during the days of onset, but in such areas *as between the folds of the buttocks, over the pubis and the points of the elbows*" (italics mine). Since cases of infantile pellagra usually have a preceding intestinal disorder, diarrhoea, dysentery, and this and the oedema keep them indoors and in bed it is not surprising that the skin lesions appear on buttocks, groins, points of elbows and knees, and over lumbar spines and not on the areas usually sun-burned. CICELY WILLIAMS quotes this article as disproving my point of view but to my mind it has stated and explained more clearly and reasonably than anything else the difference in the distribution of the dermatosis in infantile and in adult pellagra.

CICELY WILLIAMS's reference to, and picture of, a "nutritional rash" (her Fig. 6) situated as dry pigmented desquamating rash on the surface of the lower

legs of a case dying of pulmonary tuberculosis, brings one to the second article, that of LUCIUS NICHOLLS (1940) on crazy-pavement skin changes. The term crazy-pavement skin was originally used by CICELY WILLIAMS (1933) to describe the skin changes in kwashiorkor, and in that sense I have always used the word. LUCIUS NICHOLLS (1940) considers that he has found an identical change in the skin of the lower leg of many of his hospital patients in Ceylon. As infantile pellagra has probably never been reported from India and very few references and no detailed case reports appear in the literature, it is easy to understand how NICHOLLS has mistaken what most of us call mosaic skin for crazy-pavement skin. I consider that a real distinction can be made, although photographs published with my former articles have probably given rise to this impression, and appear to show a condition fairly similar to that shown in NICHOLLS's photographs.

The position has become hopelessly confused as shown by the fact that YOUNG and MALCOLM CLARK (1940) in the same journal refer to the condition as "parchment skin with hyperpigmentation" and "a mosaic appearance was frequent and often accompanied by hyperpigmentation of many of the lozenge shaped areas" over the lower legs in their cases of "hypovitaminosis." This condition is well known and consists of shining, cracked, darkened mosaic appearance of the skin over the shins and resembles cracked varnish and the hairs of this part are few or absent. It is found often in underfed and cachectic persons. It should be called mosaic skin, or parchment skin or lizard skin. It tends to desquamate but slightly and it never peels in large plaques to disclose an underlying pale area, as do the black patches of crazy-pavement. It is usually limited to the lower legs. It must not be confused with what all other writers on infantile pellagra or kwashiorkor have called crazy-pavement. LUCIUS NICHOLLS (1940) finds mosaic skin common in Ceylon, he considers that it has no relationship to pellagra, he confuses it with the crazy-pavement skin of infantile pellagra, and therefore does not consider that this disease can have any relationship to pellagra.

FIELD, PARNALL and ROBINSON (1940) discuss skin manifestations of pellagra in America, describing in great detail those types which may be overlooked. They describe areas of hyperkeratosis on the pressure sites accompanied by brown or black pigmentation. They resemble in character and distribution the black patches of crazy-pavement seen in infantile pellagra. These writers further describe mosaic skin as the ichthyosis-like changes over the shins, this is also considered pellagrous, and amenable to nicotinic acid. In this they differ from LUCIUS NICHOLLS (1940).

Since the diagnosis of infantile pellagra must depend in the present state of our knowledge on something definite I maintain that the diagnosis should never be made in the absence of these skin changes. CICELY WILLIAMS (1933) gave an excellent description, I have tried to give my own (TROWELL, 1937).

As it is necessary for workers to recognise it I venture to publish a photograph of the back and side view of a fatal case of infantile pellagra.

Briefly the dermatosis starts as jet black patches on the skin of the buttocks and the pressure areas of the back and the irritation areas of the perineum. They look as if dull black paint had been painted on to the skin, had dried and had cracked and was starting to peel off. Desquamation occurs early and reveals pale, even dead white, underlying skin. Ulceration easily occurs. Septic lesions are frequent. Fissures occur in the natural folds of the perineum, elbow, axilla, groin and behind the pinna. Generalized pallor of the skin is common. Mosaic skin can also occur. This and this alone is crazy-pavement skin and it is the hall-mark of infantile pellagra.

MANSON-BAHR (1940) has described in detail and painted in colour the changes in the glossitis of pellagra, tropical sprue, Addisonian pernicious anaemia, and idiopathic steatorrhoea, in all of which he considers there is a deficiency of the vitamin B<sub>2</sub> complex. It is clear that infantile pellagra bears a close relationship to all members of this group since it may show steatorrhoea (GILLAN, 1934; and TROWELL, 1937), and a pernicious anaemia blood picture (GILLAN, 1934; and TROWELL, 1937). Many cases of infantile pellagra show a raw red tongue having

#### CASE OF INFANTILE PELLAGRA (PLATES I AND II).

In-patient 4481/40, Mulago Hospital, Uganda. Male Mukiga child of about 7 years. Admitted 13.12.40, died 16.12.40.

*History*.—Ailing for a few months, attacks of diarrhoea, cough, oedema; and he lay indoors for a month. Dermatitis 2 weeks.

*Diet*.—Sweet potatoes, cassava and cooked plantains and small amounts of different vegetables. No meat, milk, eggs, fish, or nuts ever taken.

Parents were obviously extremely poor.

*On Examination*.—Aged about 7 years, weight 30 lb. Extreme cachexia. Oedema moderate of lower legs, and face puffy. Temp. 99.5° F., pulse 120, weak. Crazy-pavement patches on pressure sites of buttocks, outer side of thigh, knees and elbows, iliac crests, lumbar spines, ribs, and scapulae, desquamation in parts to produce pale coffee-coloured skin or dead white over lumbar ulcers. Slight generalized pallor. Lower legs showed marked mosaic skin changes, which near and above the knee became almost ichthyotic. Hair pale, scanty, thin and straight; marked cheilosis of angles of mouth, eye, nostril, and around prepuce and anus. Tongue smooth, red, raw, having no fur at all and showing enlarged fungiform papillae by the tip. Buccal mucous membrane was red, especially on the gums and between the teeth, but it did not bleed unduly. Stools were frequent, watery, greenish and contained no faecal matter. *Necator* ova were present. Few crepitations at bases. No malarial parasites in blood smears. Urine contained a small amount of albumin, and some leucocytes and a fair number of granular casts.

R.B.C., 3,300,000; Hb., 7.0 grammes; Reticulocytes, 3.0 per cent.; W.B.C., 10,000; neutrophiles, 5,000; lymphocytes, 4,000; monocytes, 600.

*Course*.—Collapse, vomiting and diarrhoea all increased.

*Treatment*.—Injections nicotinic acid 50 mg. daily for 3 days. Vomiting and diarrhoea limited intake to a few ounces of milk and egg.

*Postmortem*.—Bronchopneumonia, gut appeared thinned and the mucous membrane was atrophic, liver very fatty, kidneys were normal.

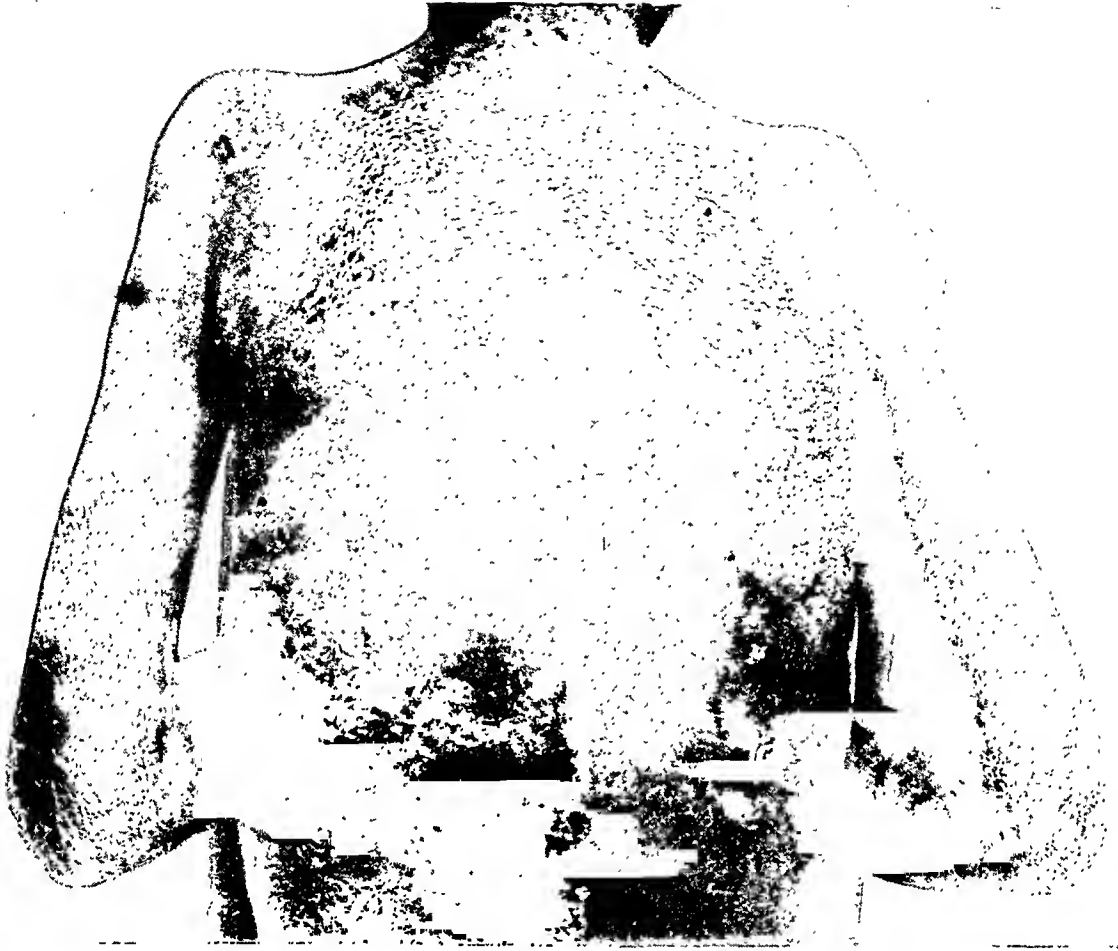


PLATE I.

FATAL CASE OF INFANTILE PELLAGRA.

Showing crazy-pavement dermatosis over pressure points of scapulae, ribs and lumbar spines, and ulceration over some of these areas.





PLATE II.

FATAL CASE OF INFANTILE PELLAGRA.

Showing desquamative dermatosis of ichthyosis-like skin over knee and elbow and surrounding mosaic-skin changes.

no fur on it and denuded of filiform papillae and presenting only a few fungiform papillae and strictly comparable to Figs. 1 to 4 in MANSON-BAHR's article (1940). Many cases show cheilosis, sodden cracked corners of the mouth and a similar condition at the corner of the nose, canthus of the eyes and around the anus and prepuce (TROWELL, 1937). The latter are regarded as definite evidence of riboflavin deficiency, and have been experimentally produced in man by SEBRELL and BUTLER (1937, 1938 and 1939). As far as I am aware no one has tried the effect of riboflavin in the cheilosis of infantile pellagra, but in view of this can it be reasonably denied that infantile pellagra is not closely associated with a deficiency of the vitamin B<sub>2</sub> complex?

If pellagra were a simple and single clinical entity with characteristic pathological findings, it would be extremely easy to prove or disprove if kwashiorkor was partly or entirely pellagra. But all attempts to prove that pellagra is a single vitamin deficiency have failed. Pellagra is not just a deficiency of nicotinic acid. SPIES (1939) recognizes in pellagra multiple deficiencies of nicotinic acid, riboflavin and thiamin. It is possible that in the future the closely related deficiency of the extrinsic factor of Castle and of protein of good biological value and of pyridoxine hydrochloride (vitamin B<sub>6</sub>) and of pantothenic acid (the chick anti-dermatitis factor) will all be noted as producing possible variations in the clinical picture of adult and infantile pellagra. We can no longer speak of the *disease* pellagra but of the *group* of deficiencies clinically grouped under the term pellagra. Infantile pellagra is certainly not entirely, and possibly not principally, a deficiency of nicotinic acid, and I have been disappointed by the treatment of the disease by nicotinic acid alone. Better results were obtained by me when marmite was employed. In the present state of our knowledge this disease appears to have closer relationship with the vitamin B<sub>2</sub> complex than with any other dietetic deficiency and in this sense it is more allied to pellagra than to any other disease.

#### SUMMARY.

There occurs largely in tropical Africa and tropical America an acute and fatal disease which commonly attacks infants in the first few years of life. It is called by a different name in almost every country. It is frequently seen shortly after weaning. It is characterized by oedema, crazy-pavement skin, diarrhoea, cheilosis and stomatitis, generalized pallor of the skin and pale, straight, scanty hair. Neurological changes may occur but are slight and are terminal. Microcytic or macrocytic anaemia and steatorrhoea are variable features. It is almost certainly due to multiple deficiency defects, the major ones being those of nicotinic acid, riboflavin and protein, the latter producing a marked nutritional oedema. All other known deficiencies of vitamins, iron and the extrinsic factor of Castle have been described in some of the cases. Possibly as yet the main deficiency is not known. Intercurrent disease is common and is frequently the cause of death and of suspected failure of treatment by dietetic measures;

bronchopneumonia, heavy malarial infection, anaemia and helminthiasis all occur. It is not known if the pellagra is the primary cause of the disease or if faulty methods of weaning and other gastro-intestinal disturbance lead to secondary pellagra, or if other infections, e.g., malaria, precipitate the vitamin deficiencies. It appears to have clear relationship with a deficiency of the vitamin B<sub>2</sub> complex, and can best be called by the time-honoured name of pellagra, since this name has for centuries described the various clinical states which characterize in all their complexity a deficiency of the vitamin B<sub>2</sub> complex. Some have thought that the oedema is due to beriberi, but there is no evidence in favour of this, and injections of the pure B<sub>1</sub> vitamin do not decrease the oedema. Those who do not accept this view may refer to the disease as kwashiorkor or the Proctor-Williams's disease.

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## PELLAGRA IN INDIA.

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" Pellagra is a disease entity characterised pathologically by an erythematous, pigmented, and exfoliative dermatitis ; stomatitis, glossitis, gastro-enteritis, hepatitis, proctitis, urethritis and vaginitis ; and clinically by the symptom syndrome of burning ( ' scalded ' ) sensation in the mouth and tongue, anorexia, diarrhoea, burning in the anal region and in the urethra, or vagina ; varied nervous and mental manifestations ; emaciation, cachexia and varying degrees of anemia." This is the comprehensive manner in which HARRIS (1939) defines pellagra. Although the work of SPIES and his associates (1936, 1938a, 1938b) has called a great deal of recent attention to this condition, nevertheless our knowledge concerning it dates back to THIERY (1755) and more especially to CASAL (1762) who described the disease under the name of " Mal de la Rosa." Since these early writings, the disease has been reported as being endemic in many parts of the world. In India the condition has usually been considered to be strictly localized (MANSON-BAHR, 1935) or to occur as rare isolated cases only (ROGERS and MEGAW, 1935). LOWE (1931), however, reported forty cases

occurring among the inmates of a leper hospital, while HARRIS (1939) has noted that a few isolated cases have been reported from India in the last few years. LOWE (1933), RAMAN (1933), RAMAN and RAU (1936), SEN GUPTA *et al.* (1939), BAJAJ (1939) and GOODALL (1940) have all reported sporadic cases occurring in Hyderabad, Andhra, the Punjab and Bengal. With the purpose of showing that the true incidence of pellagra in India has not been fully realized in the past and that the disease, in all probability, does exist in an endemic form in this country, the following cases are reported. All were recognized and treated in Miraj within the last two years and it should be stressed that they are but representative cases, chosen from a rather large series, some of the members of which are typical though most of them belong to the so-called subclinical group.

That pellagra was to be found in our wards and hence in Bombay Presidency was first drawn to our attention in an attempt to understand the following case.

### CASE 1.

Patient S. S., male, aged 40, a farmer living near Kolhapur, was admitted to the hospital on 3rd May, 1939, complaining of extreme asthenia, vague pains in the abdomen about the umbilicus unrelated to food and without periodicity, formication of the legs and feet and pigmentation of the backs of the fingers extending up the dorsum of the hands. The pigmentation had been present for 4 years while the asthenia and formication had begun 5 months previously. They were of insidious onset and had steadily become worse. He was a strict vegetarian, his diet consisting of rice, millet bread, dahl and a few green vegetables, milk only occasionally and in small quantities and no fruits. He denied any previous serious illness and his family history contained nothing noteworthy.

Physical examination revealed a well developed, fairly well nourished man who could walk only when supported on either side and then but with great difficulty. The head was normal, but there was a deviated septum and rhinitis present in the nose. The sclerae were pigmented; the pupils and the eye reflexes normal. There was much pigmentation on the tongue, more especially towards the sides. The tongue otherwise was pale, somewhat clean and atrophied. The teeth were all present and clean but there was some pyorrhoea. The pharynx was normal and the tonsils small and septic. The cranial nerves showed nothing of note. The submental glands at the angles of the jaw were large and palpable. The thyroid was normal. The chest, heart and lungs were all considered as normal. The abdomen was distended, tympanitic and there was umbilical tenderness. No fluid was present. Neither the liver nor the spleen could be felt. The extremities were normal except for some tenderness of the calf muscles on pressure. The deep reflexes, particularly those of the legs, were decreased and the knee jerks could be obtained only with difficulty. The superficial reflexes were normal. The spine and back showed nothing abnormal. The backs of the fingers were heavily pigmented. The pigment extended up the dorsum of the hands and, where it ceased, there was an erythematous edge. This edge continued to advance during the early days of his stay in the hospital and, as it advanced, the pigment followed. The skin generally was roughened, inelastic and irritative but no typical desquamative areas were present. The pulse ranged from 70 to 100 and was of low tension. The blood pressure range in hospital was 94 to 120 systolic and 60 to 80 diastolic. Assuming an erect posture did not affect it, Temperature remained normal throughout. On admission

the blood picture was as follows : R.B.C. 3,350,000, Hb. 65 per cent. Sahli, W.B.C. 7,200, polymorphs 75 per cent., lymphocytes 15 per cent., monocytes 6 per cent., eosinophils 4 per cent. The blood sedimentation was 37 mm. per hour (Westergren). The old tuberculin test, 0.01 mg., was positive. The blood Kahn was 3 plus. The fasting blood sugar was 101 mg. per cent., the blood urea 53 mg. per cent. and the blood sodium chloride 570 mg. per cent. The stool was negative for amoeba and ova and contained no cellular exudates, mucus or blood. The urine contained a faint trace of albumin and a few leucocytes. The blood smear was negative for malarial parasites.

The chest skiagram showed moderate thickening of the hilar shadows. The G-I series, following barium meal, showed a tendency to hypertonicity and increased rapidity in the advance of the barium head. Sigmoidoscopy revealed a diffuse redness of the mucous membrane only. The rectal examination was negative. Retinoscopy revealed nothing of note.

The clinical picture was considered to be probably one of Addison's disease on either a tuberculous or luetic basis and treatment was directed towards these. He was put on sodium chloride 2 drachms, and sodium citrate,  $\frac{1}{2}$  drachm daily, and given potassium iodide by mouth, bismuth twice weekly intramuscularly and neosalvarsan once weekly. Two weeks later yeast was added, 90 grains daily, but it was stopped after 3 days. On 15th June, 1939, nearly 6 weeks after admission, the patient's blood count had fallen to R.B.C. 1,580,000, Hb. 32 per cent. He was completely confined to bed and was obviously growing worse daily. Meanwhile, the blood sodium chloride had climbed to 846.5 mg. per cent., and slight oedema had appeared over the ankles. Also the patient had begun to have two to five loose motions daily and the blood pressure had dropped to 95/60. He was now quite lethargic mentally. It came to our attention that pellagra could at times resemble Addison's disease and a radical change in treatment was decided upon. All previous treatment, except the injections of neosalvarsan, was stopped, including the sodium-ion. The patient was put on cod liver oil, ferrous ammonium citrate 90 grains daily, and injections of 5 c.c. of crude liver extract. Yeast was recommenced a few days later in drachm doses. For the first time, on 27th June, 1939, the patient said he felt a little better. He refused to take liver by mouth but did consent to one egg daily. Milk, vegetables and fruits were added to his diet. Although no obvious improvement occurred in his physical status except for the control of the diarrhoea, the condition did, nevertheless, now appear to be stationary. On the 13th July, 1939, nicotinic acid\* was finally obtained and he was given 3 grains three times daily by mouth. Four days later, on 17th July, 1939, the patient was able to sit up in bed for the first time in 10 weeks. The R.B.C. had meanwhile climbed to 2,420,000 and the Hb. to 53 per cent. He continued to report himself as feeling very much better. One month later, he was able to walk about the ward without help though rather uncertainly, and on 21st August, 1939, he insisted on discharge as he felt so much improved. At discharge the R.B.C. was 4,120,000

\* The nicotinic acid used in the therapeutic trials in these cases was supplied by the Glaxo Laboratories.

and Hb. 72 per cent. During the last few weeks, the improvement in the mental condition had been marked and it was noted that the erythematous edge of the pigmentation on the hands had completely subsided. Otherwise the pigmentation remained as before but muscle tenderness and the paraesthesias had gone.

*Comment.*—A similar case to this one, characterized by extensive pigmentation, constant diarrhoea, marked asthenia and mental deterioration had been seen in the hospital wards some months before and had been treated as Addison's disease without any noticeable check in the continued downwards course that finally terminated in death. Dissatisfaction with that case and the feeling that diagnosis had probably been mistaken had lead us to sift all other possible causes of the clinical syndrome in the case reported above. The check of the downward course that occurred when a high vitamin regime was first introduced followed by the sudden dramatic upbound when nicotic acid was exhibited, leaves little doubt but that in this case we were dealing with pellagra.

## CASE 2.

V. M. P., aged 40, male, married, a clerk, was admitted to hospital on 21st December, 1939, in a maniacal condition, talking and shouting at random and requiring restraint. His family history was irrelevant. His previous history revealed an attack of malaria in 1932; and, for the past 2 years, attacks of dysentery with blood and mucus in the stools occurring about once every 2 months and lasting 2 or 3 weeks. In the intervals he was severely constipated. He had been under treatment elsewhere for this condition without effect. His mania had suddenly commenced 4 days previous to admission. He was a strict vegetarian and had been on a diet consisting of millet bread, dahl, green vegetables, tea and coffee for the preceding several months.

Examinations revealed a maniacal, restless, well developed but poorly nourished male. Eyes, ears, nose were all clear. There was a marked degree of angular stomatitis. The tongue was clear but showed patches of pigmentation along its lateral aspects; the soft palate was red and inflamed. The teeth were all present and clean but there was a high degree of pyorrhoea present. The mucosa of the lower lip was red and inflamed and showed multiple, minute ulcerations. There was also thickening and pigmentation of the nasal alar fissures. The pharynx and tonsils could not be observed because of his struggling. The submental lymph nodes were palpable. The heart, lungs, abdomen and spleen showed nothing of note. The liver was two fingers down and was tender on palpation. The extremities showed some tenderness of the muscles on pressure and, subsequently, the squatting test was shown to be positive. The deep reflexes were increased but the superficial abdominals were abolished. The skin over the backs of the finger and hands was hyperpigmented, dry, scaly, exfoliative and inelastic. Similar skin changes were present on the dorsum of the feet and toes and over the anterior tibial regions. Burning sensations and formications were subsequently complained of in the hands and feet. The pulse was rapid, 120, but of fair tension. There was a moderate degree of arteriosclerosis of the peripheral arteries.

The laboratory findings were as follows: Hb. 70 per cent., R.B.C. 4,010,000, W.B.C. 8,200, segs. 62 per cent., stabs. 5 per cent. (Schilling's classification), lymphocytes 31 per

cent., monocytes 1 per cent., eosinophils 1 per cent. No malarial parasites found. Stools contained ova of hookworm and ascaris and leucocytes. The urine was alkaline with a specific gravity of 1015, and it contained a high trace of albumin. It showed a rather high degree of pyuria. The Kahn test on the blood was negative. The spinal fluid was under normal tension and was normal. The Kahn and gum mastic test on it were negative. Blood urea was 36 mg. per cent. Blood sugar was 114 mg. per cent. Blood chlorides, done 4 weeks after admission, were 370 mg. per cent. Blood calcium was 9.4 mg. per cent.

The case was considered to be one of chronic ulcerative colitis, pyelocystitis (? amoebic hepatitis) with secondary pellagra, ariboflavinosis and thiamin chloride deficiency.

He was immediately put on injections of liver extract and nicotinic acid by mouth. The next day the mania had gone but he was still disoriented, confused and showed confabulation. On the 4th day of his treatment he was quiet and able to answer questions intelligently. A decubitus ulcer over the sacrum developed at this time but healed rapidly. The patient was also subject to furuncles which required attention in the earlier days of his hospital stay. Meanwhile the cheilosis, labial and nasal changes had all responded within a few days to the liver extract. The subsequent treatment consisted of hexamine for his urinary complaint and a course of emetin and carbarsone. The intestinal parasites were also treated by carbon tetrachloride and oil of chenopodium. One bout of malaria developed in the 3rd week of hospitalization but this quickly responded to quinine. Liver injections and liver by mouth were continued, as was also nicotinic acid. Later, yeast and cod liver oil were added. The stools, which were constipated on admission, returned to normal within 6 weeks whereas the skin lesions disappeared within the first 2 weeks. The patient, however, continued to complain of weakness and paraesthesias until the 9th week after admission when these also had gone and he requested discharge.

*Comment.*—This case is regarded as a fairly typical case of pellagra complicated by other deficiencies of the vitamin B complex and occurring in the course of a chronic diarrhoea. The response to appropriate treatment was dramatic. The patient had been on a restricted diet partly because of his feeding habits and partly because of his chronic intestinal complaint. It was thought that the additional burden of the pyelocystitis may have determined the onset of the acute pellagrous dementia. The low blood chlorides are of interest. Attempts were made to remedy this with a high sodium chloride intake in the later stages of his treatment, especially in view of the persisting weakness, but there was no clinical effect. SPIES *et al.* (1939) have recently treated this persisting weakness with injections of synthetic vitamin B<sub>6</sub> and have reported immediate results. This suggests that the clinical picture is an even more multiple one than was originally suspected. Such cases of multiple deficiency in which the pellagrous element predominates, and occurring as a complication of chronic diarrhoeal conditions, are frequently found in our wards though usually not to quite so severe a degree.



## CASE 3.

P. A., aged 38, male, Goanese waiter, was admitted to hospital on 28th July, 1939, complaining of a rash over the arms and face. This rash had appeared every April for the past 7 years and would disappear after the rainy season commenced. In later years, it had become more severe and had taken a longer and longer time to disappear until during this past year it had been present continuously, being more severe however during the hot season. He had had ulcers at the corners of the mouth and inside the lower lip from time to time. He also complained of formications and burning of the feet and hands. He admitted alcoholism but otherwise claimed his diet consisted largely of meat and rice with little or no milk, fruits or vegetables.

Physical examination was negative except for some slight pigmentation on the buccal mucosa of both sides of the mouth, pyorrhoea, and hyperreflexia. The skin over the dorsal aspect of both the hands and forearms to just above the elbow was dry, scaly and desquamative, and there was a serous discharge from the backs of the hands. There was hyperpigmentation of the dorsal areas of the terminal phalanges of the fingers and toes. The skin over the ears, about the neck and sides of the face also was desquamative, dry and scaly but there was no serous discharge from these areas. The laboratory findings, including serology, showed nothing of note.

The patient was put upon nicotinic acid, 10 grains daily, and yeast. Improvement was immediate and steady. Within 1 month the rash had completely cleared from the face, ears and neck and, 2 months after admission, the arms and hands were also healed and the patient was discharged as cured. No other medication was employed.

## CASE 4.

N. P. P., aged 35, male, a farmer from Berar, was admitted to the out-patient department on 18th August, 1939, complaining of rash over the dorsum of both hands and both feet, the back of the forearms to the elbow and over the lower two-thirds of the anterior surfaces of the legs. The rash had first appeared 2 months earlier on the back of the left hand and had quickly spread from there to involve the areas stated and the ears, and had continued to spread despite treatment for eczema and neosalvarsan injections. He admitted having had ulcerations at the angles of the mouth and a burning, sore tongue from time to time during the past few years. He denied alcoholism or syphilis. He was a strict vegetarian and his diet consisted mostly of millet bread, dahl, rice and a few green vegetables.

Physical examination revealed stomatitis on the inside of the lower lip, pyorrhoea and hyporeflexia. The rash was exfoliative, desquamative, dry mostly and scaly but with rather marked weeping over the dorsum of the hands and feet. It also involved the ears and backs of the ears. There was increased pigmentation of the areas involved, more so over the dorsal areas of the terminal phalanges of the fingers and toes. There was a slight oedema about the ankles. Paraesthesias were denied and there were no other complaints. The laboratory findings revealed a moderate degree of secondary anaemia only.

Only calamine lotion was used locally and the patient was given yeast, iron and nicotinic acid 10 grains daily, by mouth. Improvement was immediate on this regimen. A more varied diet was also advised. On 27th September, 1940, only the dorsum of the feet and the backs of the hands still showed lesions and the patient asked for discharge promising to continue treatment at his own home. Two weeks later he reported himself as completely cured.

*Comment.*—Cases 3 and 4 are reported as examples of the skin lesions of pellagra. In the former case alcoholism probably played a part in causing the development of the lesions but, in the latter case, no primary pathology could be discovered and the case must be considered as true endemic pellagra. The absence of the other two members of the triad, namely diarrhoea and mental symptoms, is notable.

#### CASE 5.

N. H. P., aged 30, male, a farmer, was admitted to the hospital on 19th February, 1940, complaining of a painful tongue and throat and with substernal burning, all made much worse by eating spices. These symptoms had been present for the past 3 weeks and were becoming worse. For the past 2 years he had had attacks of dysentery when he would pass frequent motions containing mucus and blood, severe constipation being present in the intervals. These diarrhoeal attacks had been more frequent during the past 6 months. The last 2 to 3 days preceding admission the tongue has been so painful he could take no food at all. He was a strict vegetarian and, because of his dysentery, for the past year his diet had consisted only of rice, sago, with green vegetables and fruits but occasionally.

Physical findings showed the tongue to be fiery red, clean, atrophied, smooth and glistening. The pharynx and soft palate were hyperaemic. The abdomen showed slight hypogastric tenderness only. The skin over the dorsum of hands and feet, over the backs of the forearms and the anterior surfaces of the legs and over the lower parts of the face, was dry, scaly, thinned and inelastic and showed areas of the "crazy-pavement" type of lesions. These areas also showed increased pigmentation, again more marked on the backs of the fingers and toes.

The laboratory findings revealed a severe degree of secondary anaemia and there were both cystic and motile forms of *Entamoeba histolytica* in the stools. Other laboratory findings, including serology, were negative. No diarrhoea occurred while in hospital.

Treatment consisted only of iron and bismuth with the addition of milk, orange juice and liver to the diet. This was the only treatment for the first week, and no improvement resulted. On 2nd March, 1940, yeast was added to the diet with cod liver oil. Nicotinic acid was begun by hypodermic injections. The next day the patient reported considerable improvement in the tongue condition. The above regimen was continued with daily injections of nicotinic acid, until 9th March, 1940. At this time the patient stated that all subjective symptoms in the tongue and throat had disappeared although no changes in their outward appearance were evident. Anti-amoebic treatment was now started but on 11th

March, 1940, the patient felt so much improvement that he demanded discharge. This was granted against advice.

#### CASE 6.

H. S., aged 25, male, farmer from Cutch, was admitted to the hospital on 1st March, 1940, complaining of a sore and burning tongue, burning in the throat, substernal and rectal burning and burning micturition. Spicy foods made the mouth and throat symptoms much worse. Two years previously he began to have two or three loose motions daily with slight evening fever. These had persisted. One year ago he had first noticed the burning sensations and these had so increased in severity as to now constitute his chief complaint. He was a strict vegetarian and his diet had consisted of rice, dahl, ghee, and occasionally a few green vegetables and fruit not oftener than once weekly.

The physical examination revealed a red, atrophied, clean, tender tongue, with hyperaemia of the mucosa of the inner aspects of both upper and lower lips and of the buccal surfaces. Small, pin-point, whitish, shining papules could be seen on the inner aspect of the lower lip. These were very tender. The soft palate and pharynx were also hyperaemic. Pyorrhoea was present. There was a thickening of the alar folds with desquamation of the alae nasi and with multiple seborrhoeiform accumulations extending laterally as far as the malar prominences and almost of a butterfly shape. The skin of the dorsum of the hands and feet was thinned, inelastic and cracked and showed hyperpigmentation. There was hyperreflexia and the calf muscles were tender.

The laboratory findings showed a moderate degree of secondary anaemia, an increased blood sedimentation rate, normal urine and a normal serology. The old tuberculin test, 0.01 mg., was positive 3 plus. Examination of the stool by the anti-formin concentration method for tubercle bacilli showed it to be positive, Gaffky 6. The skiagram of the chest revealed nothing of note. The diagnosis was that of tuberculous enterocolitis with secondary nicotinic acid and riboflavin deficiencies and the Plummer-Vinson syndrome.

The patient was put on iron and nicotinic acid by mouth and nicotinic acid by hypodermic injections. The tongue symptoms were immediately reported as being much relieved. Milk, liver, tomato and orange juice were added to the diet and, on 14th March, 1940, liver extract injections, 2 c.c., were begun every second day. Ultra-violet light to the abdomen was commenced on 11th March, 1940, with cod liver oil by mouth. Nicotinic acid by injection was discontinued at this time. On 19th March, 1940, the patient complained that his tongue symptoms were recurring despite nicotinic acid still being given by mouth and no other changes having been made in the above routine. Nicotinic acid by hypodermic injection was recommenced. On 14th March, 1940, a greyish coating had appeared over the central area of the tongue. On 28th March, 1940, the tongue was still smooth but of a normal colour and not painful. Diarrhoeal attacks continued off and on during his hospital stay but on 28th March, 1940, he claimed to be very much improved and asked for discharge. This was granted against advice. At discharge the skin lesions about the nose also had completely disappeared and there were no mouth complaints.

*Comment.*—These cases are illustrative of the tongue lesions of pellagra. Both patients complained much of the severe generalized burning of the nicotinic

acid injections which lasted up to 1 hour. Another patient not reported here complained of this burning on receiving the drug by mouth. The response to nicotinic acid in all our tongue cases has been immediate and each time the patient has shown so much improvement as to demand discharge before we have considered him ready for it. The sore tongue, the difficulty in swallowing and the associated anaemia in these cases has given us a picture highly suggestive of the Plummer-Vinson syndrome. The similarity also to the mouth symptoms of sprue is remarkable. It is suggested that both these may be but varying degrees of nicotinic acid avitaminosis. The lack of absorption of nicotinic acid from the intestinal tract was quite apparent in Case 6.

### CASE 7.

S. H., male, Christian, aged 40, and a ward boy in this hospital, was admitted to the wards on 23rd December, 1938. The preceding 2 days he had felt feverish and had attended the out-patient department where he had received treatment for a common cold. On the day of admission, in the morning, he suddenly became maniacal, showing complete disorientation and confabulation. He had attempted to do violence to those around him and his struggles were so severe as to require four men to restrain him. The patient was known to suffer from chronic colitis of a low grade for which he had received treatment from time to time.

Physical examination showed a poorly nourished male with pyorrhoea and hyperpigmentation. The skin over both tibiae was shiny and thin, the "crazy pavement" effect being well seen over the face, hands, arms, legs and feet. The rest of the examination was essentially negative. Laboratory findings showed a moderate degree of secondary anaemia, amoebic cysts in the stool and negative serology. The spinal fluid findings, including the gum mastic test, were also negative.

The patient was immediately put under restraint and heavy doses of sedatives ordered. These resulted in control of the maniacal symptoms while he was under their effects but no improvement in the mental condition occurred. On 25th December, 1938, the patient's condition was definitely worse and he was showing signs of exhaustion. The next day, large doses of liver extract were begun intramuscularly and nicotinic acid was given by mouth. The effect was dramatic. On 27th December, 1938, the patient was quiet and subdued without sedatives and, on 28th December, 1938, he was essentially normal in his mental processes. The treatment was continued and 1 week later he was discharged as mentally well and declaring that he also felt much better physically. Dietary instructions given at that time have been followed and the patient has remained well since. Subsequently it was learned that, preceding the onset of his illness, he had been on a very restricted diet of rice with a little millet bread only.

*Comment.*—This patient illustrates a type of case that is seen in the wards of this hospital from time to time. Not all of them respond so dramatically to the effects of the vitamin therapy but about one-third of the cases met in our

rather limited experience have done so. In consequence, we have concluded that those that do so respond represent an acute maniacal type of pellagra in which the dermal and intestinal manifestations of the disease are minimal or absent. In two other cases seen, one a girl suffering from an extensive bilateral pulmonary tuberculosis with intestinal ulceration and the other a case of chronic bacillary dysentery, nicotinic acid therapy produced considerable improvement but not a complete remission of the mental symptoms. This was due, in all probability, to the fact that in neither case was the intestinal condition controlled.

### CASE 8.

D. A., male, aged 7—his father a poor farmer and he a strict vegetarian—was admitted to the hospital on 9th March, 1940, complaining of ascites, puffiness of the face, generalized weakness and apathy, burning of the tongue and throat, formication and burning of both hands and feet, burning urination and anorexia. His younger brother, aged 4, was admitted at the same time with practically identical symptoms though the mental apathy was not so marked. The present complaint began about 2 months previously with a bout of fever. At the same time it was noticed that skin changes had occurred that these were much worse on exposure to the sun. The patient felt cold and chilly all the time and would sit in the sun well protected by clothing for hours at a time. The previous history was negative except for some occasional bouts of fever. His diet, before the onset of anorexia, consisted of rice, dahl, a little millet bread and chapatti, vegetables once weekly only, no fruits, and about half a cup of milk daily. During the last 2 months before admission he would take only small quantities of milk.

Physical examination revealed a very apathetic, pale and markedly anaemic boy, showing considerable pyorrhoea, a pale, almost white, and glossy tongue, a slightly enlarged heart with poor muscle quality in the cardiac sounds but no murmurs, an abdomen distended with fluid, slight pre-tibial pitting and hyporeflexia. The face was pale and puffy. The skin of the face, neck, arms as far as the elbows, backs of hands and fingers, the anterior aspects of the legs and the dorsum of the feet was thin, inelastic, dry, glossy, desquamative and cracked and with a well defined "crazy pavement" appearance. The muscles of the calves of the legs were intensely tender. The splenic pole was at the umbilicus and the liver was three fingers down from the costal arch.

The blood picture was as follows : Hb. less than 20 per cent. (Sahli), R.B.C. 750,000, W.B.C. 4,800, segs. 39 per cent., lymphocytes 61 per cent., smear negative for malarial parasites. The blood sedimentation rate (Westergren) was 39 mm. Serum proteins were above 6 per cent. The old tuberculin test, 0.01 mg., was positive. Kahn test was negative. Urinalysis was negative. The stool showed ascariasis and ankylostomiasis at different times, both being slight infestations. Agglutination test for *B. dysenteriae* were negative and stool cultures for the *dysenteriae* organisms were also negative. The skiagram of the chest was negative and the stool was negative for the tubercle bacillus by concentration methods. The patient ran a low grade fever on admission as well as two to three loose stools daily.

He was immediately put on cod liver oil, nicotinic acid, ferrous ammonium citrate in large doses and a balanced diet supplemented by liver, eggs and orange juice daily. Liver extract was given intramuscularly twice weekly and the

intestinal parasites were treated. The fever responded in a few days to cinchona by mouth. Two weeks after admission it was noted that the skin of the face and neck had cleared by desquamation and the child had acquired a ravenous appetite and would now take an interest in the events of the ward. The ascites had disappeared by 28th March, 1940, and the diarrhoea had stopped. Yeast was then added also to the above regimen. The tongue complaints disappeared shortly thereafter and, on 5th April, 1940, the child was up and alert, the skin changes on the arms and legs were clearing rapidly and the Hb. was 40 per cent. and the R.B.C. 2,100,000. Muscle tenderness was also less. Progress thereafter was less rapid but at discharge, on 13th May, 1940, the skin lesions had completely cleared, the child appeared mentally normal for his age and was about playing with other children, his Hb. was 50 per cent., the R.B.C. 3,140,000 and muscle tenderness was gone. The boy was subsequently seen on 15th June, 1940, and then still appeared anaemic and with a puffy face but otherwise quite normal. Neither the spleen nor the liver was then palpable.

*Comment.*—TROWELL (1940) has described the syndrome of infantile pellagra as seen in Africa. The case reported above is regarded as belonging to this group although we believe that other vitamins, notably thiamin, were also deficient. A marked iron deficiency anaemia and chronic malaria also played their part. The intestinal parasites were probably only incidental. It is interesting to note that the younger brother, who was also in the hospital at the same time, suffered from the same complaints although to a lesser degree, and showed definite pellagrous lesions on the backs of the hands. The father of these children some weeks later also was admitted to the ward suffering from a chronic diarrhoea with severe anaemia and oedema of the legs. This also responded to treatment identical with that given to the son. In addition, however, the father showed definite impairment of kidney function which improved with lessening of his anaemia.

#### CASE 9.

K. P., aged 40, housewife, a strict vegetarian, was admitted to the out-patient department on 1st March, 1940, complaining of burning in the tongue and throat on eating spices, of burning urination, and stating that the skin becomes sore and tender on exposure to the sun. The diet contained no meat, milk or fruit. Daily she took rice, some millet bread and a small helping of green vegetables only.

Physical examination showed a clean, red, sore tongue, pyorrhoea alveolaris and some tympanites and borborygmus in the abdomen. The skin was thin, inelastic, dry and scaly over the backs of the hands and forearms, the dorsum of the feet and the lower anterior tibial areas. Laboratory findings revealed a moderate degree of secondary anaemia and there were amoebic cysts and vegetative forms in the stool. The urine was clear.

The patient was put on nicotinic acid solely and rapid clearing of the tongue, skin and urinary symptoms occurred within the week. Skin signs remained

unchanged, however, in that time. Later the amoebic infection was treated and iron given for the anaemia.

On completion of the anti-amoebic treatment the patient pronounced herself as feeling fit and asked for discharge from the clinic. She has since returned to the clinic on several occasions for other minor complaints and has stated that she remains clear of all her former complaints but does have paraesthesias of the hands and feet.

*Comment.*—This case illustrates a common clinical problem in our out-patient clinic and such cases are classified as sub-clinical pellagra. Response to nicotinic acid is usually a matter of a few days only but as a rule the patients do not persist in treatment long enough to result in a complete clearing of the skin changes. Their frequent complaints of paraesthesias and formication would indicate the multiple nature of the deficiency in these cases also.

#### CASE 10.

T. S., male, aged 22, Christian, was first admitted to the out-patient department on 6th June, 1939, complaining of an irritable, papular rash over the glans penis, and burning micturition.

Physical examination was negative except for a number of papules on the glans. He denied venereal disease and smears for the gonococcus and serology were both negative. The urine showed nothing abnormal.

Treatment for scabies, local applications, urethral irrigations and many other remedies were all without effect and both the patient and the attending physician became greatly discouraged. The patient however persisted in returning to the clinic at regular intervals. On 8th March, 1940, he began to complain of burning in the tongue on eating spices. He was at once put on yeast and nicotinic acid, all other medication being stopped. Ten days later the patient reported that the burning in the tongue and in the urethra had disappeared and the papular rash on the glans penis had healed. He asked for and obtained discharge from the clinic and since has reported that there has been no recurrence of his symptoms.

*Comment.*—This case is reported as another example of the subclinical type that went undiagnosed for nearly one year. The immediate response of the tongue and urethral burning to the treatment given indicates that they were pellagrous but, beyond stating that the response of the papular penile rash to this treatment would indicate its connection to a B-complex deficiency, the cause of this last is unknown.

#### DISCUSSION.

Since it has been customary to present to medical students through their textbooks and lectures, only the classical picture of the vitamin deficiencies, it is little wonder that the milder and more confused forms have been consistently mis-diagnosed and the real incidence of these conditions therefore not appre-

ciated. This failure has been due to the unfamiliarity of the average practitioner with the manifestations that pellagra, and for that matter thiamin deficiency also, will present in its partially developed state; and also because they have not realized that most cases are complicated by other deficiencies of the vitamin B complex and produce a picture that is frequently one of great variability. SPIES *et al.* (1940) have also stressed this multiplicity of the usual vitamin deficiency clinical picture. MACKIE, EDDY and MILLS (1940) have stated :—

“Nicotinic acid deficiency, therefore, is directly linked to acute glossitis, stomatitis and enteritis. Characteristic changes occur in the skin especially in those areas exposed to sunlight. Acute dermatitis appears first, followed by pigmentation and atrophy which leaves the skin thin and parchment-like. Similar changes, although usually less intense, may occur in the peri-anal region, the scrotum, vulva and vagina.”

This statement of the condition is a very fair one as we observe it in this hospital. The pigmentation and the atrophied, parchment-like skin are tell-tale signs observed with marked frequency, the glossitis and the stomatitis only less so. Acute pellagrous erythematous dermatitis is seen from time to time but apparently the patients do not usually seek help at this stage. Chronic diarrhoeas are so frequent in our wards and other possible causes of the diarrhoea so often manifest that we still find difficulty in being certain when a nicotinic acid deficiency is the cause of this symptom. The classical triad of diarrhoea, dermatitis and dementia has presented itself in the same patient only on occasion.

The majority of our cases have been considered as secondary to another pathological condition. The primary diagnosis has usually been chronic bacillary dysentery, chronic amoebic dysentery, tuberculous entero-colitis or idiopathic ulcerative colitis. A number have also been associated with hookworm anaemia and with chronic malaria but, as a rule, these last have shown rather pronounced evidences of a thiamin deficiency, the pellagrous element frequently being the minor one. The question of the influence of severe anaemia, secondary or primary, on the development of vitamin B deficiencies, particularly thiamin deficiencies, should be investigated. Of the primary pellagrins seen the majority come during the cold and hot seasons, the rainy season being conspicuously void of them. Furthermore, our most severe cases have been in vegetarians, a fact that has also served to complicate their treatment and the correction of their diets since milk still remains a luxury to the average Deccan villager.

### Conclusion.

Pellagra, mostly of a secondary type, is a common condition in the Bombay Deccan.

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FURTHER NOTE ON A METHOD OF STAINING MALARIAL  
PARASITES IN THICK BLOOD FILMS.

BY

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In an earlier communication I described a simple, rapid and apparently reliable method of staining thick blood films for malaria (FIELD, 1940). The method, developed originally on lines suggested by the work of SIMONS (1938), and PAMPANA (1938), finally introduced what was believed to be a new idea in blood staining—the use of the haemoglobin to provide colour contrast and, in effect, to serve as a counter-stain. SIMONS had drawn attention to the

\* My assistant, Mr. YAW WAH CHEW, has tested the effects of a variety of stains on dried blood and the final working out of a practical thick-film staining method for rapid malarial diagnosis was much facilitated by his observations.

My thanks are also due to Mr. R. A. WRIGHT, Veterinary Officer, Malacca, for the supply of trypanosome-infected blood.

extremely rapid penetration of certain basic stains to leucocytes and blood protozoa; PAMPANA had stressed the desirability in thick film staining of avoiding osmotic stresses by using aqueous solutions of stain isotonic with blood plasma. With the observations of these workers as a point of departure a method was evolved by which thick blood films could be stained in one second in such a manner that stained parasites and leucocytes were contrasted against a background of laked haemoglobin. The method had the advantages of simplicity and speed and was shown to compare not unfavourably with other thick-film staining methods in the facility and accuracy with which a diagnosis of malaria could be made. The two main drawbacks were the pallor of chromatin and the prominence of the reticular and chromatoid material of immature red cells; both due to the use of a single stain with a selective affinity for cytoplasm and for red cell reticulum, but little for the chromatin.

The stain has now been in routine use in this laboratory for over a year and its utility, at least for certain requirements of malaria research, has been confirmed. ROBERTS (1940) in East Africa has adopted the stain for routine diagnosis and reports that, in the hands of African laboratory assistants, the speed and simplicity of the method are consistent with accuracy. Meanwhile, it has been found possible while retaining the basic features, to adapt the method to the Romanowsky principle and to stain the chromatin differentially in an actual staining time of little over 2 seconds. The brilliant cresyl blue originally used has been replaced by a mixture of methylene blue and methylene azure and the films are counterstained with eosin. This paper describes the present procedure.

#### THE ROMANOWSKY EFFECT.

The chromatin of malarial parasites is only faintly stained by methylene blue. Counterstaining with eosin has little further effect. ROMANOWSKY in Russia and MALACHOWSKY in Germany, in the early years of the century discovered, almost simultaneously, that the effects of these two stains are modified if the methylene blue is exposed to the action of alkali or is matured with age; the chromatin then stains a deep red. (MOSKOWSKY, 1935; KINGSLEY, 1937). This effect was soon shown to be due to decomposition products within the methylene blue, the "rot aus methylenblau" of German authors, now usually termed methylene azure. The staining of the chromatin, according to GIEMSA (1922), is a dual effect of the methylene azure and the eosin; the basic azure mordants the chromatin and the acid eosin stains it. Mixtures of methylene blue and eosin were in use before ROMANOWSKY's discovery, but with these mixtures the chromatin of malarial parasites stained blue and was thus undifferentiated in colour from the cytoplasm. GIEMSA was able to isolate methylene azure from solutions of prepared methylene blue. He called the isolated substance Azure I. A mixture of equal parts of Azure I and methylene

blue he called Azure II. The original Romanowsky stains and their many variants all contain methylene blue, methylene-azure and eosin: the methylene blue stains the cytoplasm of malarial parasites, the methylene azure-eosin stains the chromatin.

#### THE ROMANOWSKY PRINCIPLE APPLIED TO RAPID STAINING WITH ISOTONIC AQUEOUS SOLUTIONS OF STAIN.

The main defect of our original staining method was the poor definition of the chromatic dots of *falciparum* 'ring' forms. We had tried to correct this defect by counterstaining with known chromatin stains but without success. A few months ago, when we had abandoned the attempt to stain chromatin and had accepted the failure as inherent in the methods we were using, one of my assistants, Mr. YAW WAH CHEW, showed me a thick film from a case of *falciparum* malaria, stained by the same methods that had failed a year before but with the chromatin dots stained a deep purple-red. The film had been exposed to an isotonic solution of methylene blue for 1 second and to isotonic eosin for another second. The methylene blue solution was now a year old. The suggestion thus arose that the chromatin staining was a true Romanowsky effect due to the development of methylene azure with ripening of the stain. That this was probably the explanation we were able to show by repeating our failure of the previous year with freshly prepared solutions; and by obtaining consistent chromatin staining when, to freshly prepared methylene blue solutions, we added a small quantity of Azure I. By the technique we now use thick blood films are dipped for 1 second successively into jars containing methylene blue-azure and eosin in isotonic solution, with a rinse in water for a few seconds in between. The following notes on technique supplement and amend those given in the earlier paper.

#### TECHNIQUE.

*Preparation of Films.*—The blood films should be about the size of a shilling and *not too thick*; a thickness of from ten to fifteen times that of thin films is enough; the dried thick film should not be so thick that the hands of a watch cannot be seen through it. The films may be made by any of the conventional methods. The films are ready to stain as soon as they cease to be obviously moist. Drying may be accelerated with a hot-air current from a hair-dryer. Fixation is unnecessary. Freshly prepared films stain better than films which have been kept for a day or two.

*Preparation of Stain.*—Two solutions are used—methylene blue-azure and eosin, both in isotonic solution adjusted to pH 6.6. Isotonicity and correct pH are determined by the amount and proportions of the acid and alkaline phosphates which the stains contain.

Solution (A)	Methylene blue †	..	..	..	..	0.8 grammes
	Azure I *†	..	..	..	..	0.5 "
	Disodium hydrogen phosphate (anhydrous)					5.0 "
	Potassium dihydrogen phosphate (anhydrous)	..	..	..	..	6.25 "
	Distilled water	..	..	..	..	500 c.c.
Solution (B)	Eosin †	..	..	..	..	1.0 grammes
	Disodium hydrogen phosphate (anhydrous)					5.0 "
	Potassium dihydrogen phosphate (anhydrous)	..	..	..	..	6.25 "
	Distilled water	..	..	..	..	500 c.c.

The phosphate salts are first dissolved, then the stain is added. Solution of the granular Azure I is aided by grinding in a mortar with a small quantity of the phosphate solvent. The solutions of stain should be set aside for 24 hours when, after filtration, they are ready for use. Should a scum later appear on the surface, or dye precipitate on the stained films, subsequent filtration is necessary.

The same solution may be used continuously for many weeks without apparent deterioration but the eosin solution should be renewed when it becomes greenish from the slight carry-over of the methylene blue.

The stains are kept in covered jars of such a size that the depth of solution is about 3 inches, the level being maintained by the addition of fresh stain as necessary.

### *Technique of Staining.*

- (1) Dip the film for 1 second into Solution (A).
- (2) Remove from Solution (A) and immediately rinse by waving *gently* in clean water for a few seconds until stain ceases to flow from the film and the glass of the slide is free from stain.

\* The American equivalent of the German Azure I is Azure B, not as has been sometimes wrongly supposed, Azure A. (ROE, M. A. and co-workers, 1940.)

Should Azure I be unobtainable it is possible to prepare a methylene blue-azure mixture of undefined composition from medicinal methylene blue. I am indebted to Mr. A. V. HITCH, Pharmaceutical Chemist of the Perak Medical Department, for the following simple method of producing a satisfactory solution, Solution (A), from methylene blue and buffer phosphate salts.

- i. Dissolve 1.3 grammes of medicinal methylene blue and 5.0 grammes of anhydrous disodium hydrogen phosphate ( $\text{Na}_2\text{KPO}_4$ ) in 50 c.c. of distilled water.
- ii. Bring to the boil and then evaporate in a water bath almost to dryness.
- iii. Add 6.25 grammes of anhydrous potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ).
- iv. Add 500 c.c. of distilled water, stir till the stain is completely dissolved and set aside for 24 hours.
- v. Filter before use.

† Medicinal methylene blue Azure I : Supplied by G. T. Gurr, of London.

Yellow eosin, water soluble :

Supplied by British Drug Houses, London.

- (3) Dip for 1 second into Solution (B).
- (4) Rinse by waving *gently* for 2 or 3 seconds in clean water.
- (5) Place *vertically* against a rack to drain and dry.

The concentration of the stain is adjusted for staining times of 1 second with an immediate wash of 5 seconds, but the relative times may need slight adjustment to suit different batches of stain. Varying periods of from 1 to 5 seconds should be tried until the results are optimal for the particular stains and washing water in use.

### *Colour Differentiation.*

Staining is optimal at the lower edge of the film towards which the haemoglobin has drained. In this area the colour differentiation is as follows:—

*General ground* : creamy-yellow colour, sometimes uniform, sometimes mottled with pale blue.

<i>Leucocytes</i> .. ..	Nuclei :	Deep blue, sharply defined.
	Cytoplasm :	Pale blue, vaguely defined.
	Granules :	Eosinophilic, large, dull red, well defined.
		Neutrophilic, small, pale purple, vague.

<i>Malarial Parasites</i> ..	Cytoplasm :	Blue.
	Chromatin :	Dark purplish-red.
	Pigment :	Unstained yellow of varying shades depending on the depth of the cytoplasm in which it lies.

### COMPARISON WITH GIEMSA STAIN.

The clarity of the blood picture in films stained by this rapid method compares not unfavourably with that of Giemsa-stained films. The differences in the two methods and in their staining qualities are summarized in the accompanying table (p. 40).

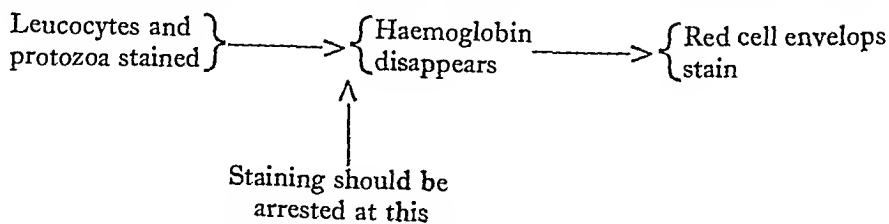
### COMMENTS IN GENERAL PRINCIPLES.

Certain basic stains in isotonic solution are able to penetrate dried blood films not more than  $50\mu$  thick with great speed and are rapidly adsorbed to leucocytes and the cytoplasm of blood protozoa. This is the first, almost instantaneous, effect. Thereafter they dissolve out the haemoglobin, and finally stain the red cell envelopes. So long as the haemoglobin remains, the red cells have little tendency to take the stain. If, now, the concentration of the stain and the time of staining are mutually adjusted so that the process is arrested at the point where leucocytes have stained, where the red cells are laked but much of the haemoglobin remains and the red cell envelopes have

### COMPARISON OF THE GIEMSA AND RAPID ISOTONIC METHYLENE BLUE-AZURE-EOSIN STAINING METHODS FOR THICK BLOOD FILMS.

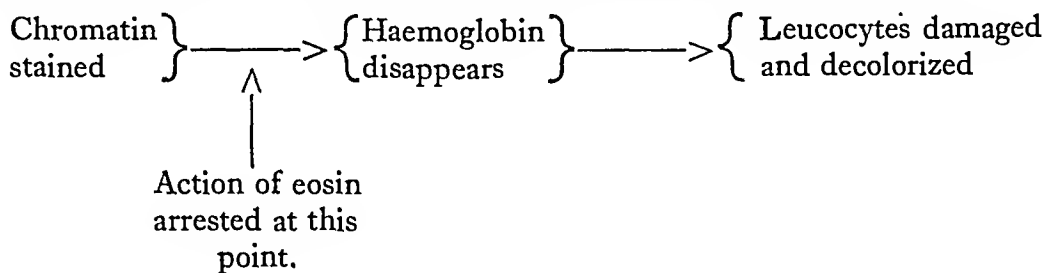
	Giemsa.	Isotonic methylene blue-azure-eosin.
Time of drying of film...	Several hours—at least 8 in the moist tropics	1-10 minutes depending on the dryness of the atmo- sphere
Time of staining exclud- ing drying of film	20-60 minutes	Less than 10 seconds
Film background ...	Mottled pale grey to blue grey	Uniform creamy-yellow to mottled blue-grey depend- ing on the amount of residual haemoglobin
Leucocytes ... ..	Well stained but cyto- plasm much tattered and granules often lost ; nuclei often distorted	Better preserved ; nuclei in- tact, cytoplasm and granules usually retained
Platelets ... ..	Appearances similar	
Reticulum and nuclear debris of immature erythrocytes	Lightly stained but not usually obtrusive	More deeply stained and liable to give appearances which are confusing in malarial diagnosis
Malarial parasites :		
i. General ... ..	Seen on a pale clean ground—blue against pale-grey	Seen on a pale ground clean except in anaemia—blue against creamy-yellow—a beautiful colour contrast in appropriate parts of the film
ii. Young tropho- zoites	Good colour distinction between chromatin and cytoplasm. Rings usually collapsed	Chromatin and cytoplasm both well stained. Ring form more often preserved
iii. Older tropho- zoites, schizonts, gametocytes	Well stained but often somewhat eroded	Well stained ; less erosion of outline but internal struc- ture less evident

not yet begun to stain, a selective effect is obtained ; leucocytes, platelets and protozoa alone take the stain and lie on an unstained or very lightly stained ground which is yellowish from retained haemoglobin. The sequence may be illustrated as follows :—



But basic stains used in this manner do not give good definition of chromatin. Chromatin must be counterstained in a contrasting colour. The usual chromatin stains, including eosin, alone have little effect, but eosin used after mordanting with methylene azure has a strong selective affinity for chromatin—the Romanowsky effect—and when the basic stain used is methylene blue the range of colour is that of the conventional Romanowsky methods.

The eosin counterstain must be used with care. Solutions of eosin at a concentration appropriate for ultra-rapid staining tend to damage and decolorize unfixed leucocytes stained with methylene blue-azure. Moreover, when the application is prolonged beyond a few seconds they accelerate the disappearance of the haemoglobin. What is required is that the chromatin shall be rapidly stained, that the leucocytes shall retain their blue colour and that at least a part of the haemoglobin shall remain. To obtain this effect the action of the eosin must be interrupted at the point indicated hereunder.



The stage in the staining process at which chromatin is stained but cytoplasm is not yet decolorized is reached in about 1 second when a 0.2 per cent. concentration of eosin is used on films previously exposed for a similar period to an isotonic methylene blue-azure solution of similar strength.

Well stained films prepared by these methods show a beautiful play of colour best shown at the stage towards which the haemoglobin drains. The effect is tri-chromatic—blue cytoplasm and deep purple chromatin on a cream ground. With non-anaemic blood the ground is clean and, if not uniform, not more than pleasingly mottled with pale blue.

The only significant drawbacks yet encountered have been in anaemic blood. The chromatoid and reticular residues of immature erythrocytes stain more obtrusively than with Giemsa stain and may confuse an inexperienced worker. Chromatoid dust derived from degenerate or immature red cells may be mistaken for the chromatin of malarial parasites. Lysed reticulocytes appear as purple-stained "clouds" which may have a fortuitous resemblance to malarial parasites, particularly if accompanied by chromatoid material. Severe anaemia, furthermore, interferes with the rate of penetration of the stains. Normally an exposure of 1 second to each stain is ample for rich deep staining but for reasons which we have not been able to explain the stains penetrate



more slowly when the haemoglobin content of the blood is much reduced and an exposure of 10 seconds or more to each stain may then be necessary.

### SUMMARY.

Observations are made on a simple method of staining malarial parasites in thick blood films which :

(a) Does not require prolonged drying of the blood.

(b) Produces the Romanowsky effect in a total staining time of less than 10 seconds.

(c) Is tri-chromatic with sharp colour contrasts between parasites and the ground of laked red cells on which they lie.

(d) Damages leucocytes and blood protozoa less than do the standard methods of Giemsa staining.

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FIG. 1.—Showing the sharp definition of the chromatin dot.



FIG. 2.—Showing the retention of the "ring" form. Phagocytosis of a young schizont by the large mononuclear cell is also clearly evident.



FIG. 3.



FIG. 4.

# PLATE I.

PHOTOMICROGRAPHS OF YOUNG *falciparum* TROPHOZOITES IN THICK BLOOD FILMS STAINED FOR ONE SECOND WITH ISOTONIC METHYLENE-BLUE-AZURE AND COUNTERSTAINED FOR ONE SECOND WITH ISOTONIC EOSIN ( $\times 1,700$ ).



FIG. 5.—Segmenting *P. vivax* with one trophozoite, two polymorphs and one lymphocyte, showing preservation of general form.



FIG. 6.—Gametocytes of *P. falciparum* with four leucocytes and a dense group of blood platelets.



FIG. 7.—Gametocytes of *Plasmodium* sp. from Malayan flying-fox ; with two trophozoites and two polymorphs.



FIG. 8.—*Trypanosoma* sp. from Malayan buffalo ; with one polymorph (experimental infection in guineapig).

## PLATE II.

PHOTOMICROGRAPHS OF MALARIAL PARASITES OR TRYPANOSOMES IN THICK BLOOD FILMS STAINED FOR ONE SECOND WITH ISOTONIC METHYLENE BLUE-AZURE AND COUNTERSTAINED FOR ONE SECOND WITH ISOTONIC EOSIN ( $\times 1700$ ).

FIG. 9.—Giemsa-stained thin blood film showing anisocytosis from severe anaemia.

FIG. 10.—Thick blood film from same case rapidly stained with isotonic methylene blue-azure-eosin. The reticular material of immature erythrocytes produces blue "clouds" which may sometimes confuse diagnosis.

FIG. 11.—Giemsa-stained thin blood film showing punctate basophilic degeneration of erythrocytes.

FIG. 12.—Thick blood film from the same case rapidly stained with isotonic methylene blue-azure-eosin. Chromatoid dust derived from the erythrocytes may sometimes be mistaken for the chromatin dots of malarial parasites.

### PLATE III.

PHOTOMICROGRAPHS OF RETICULAR AND CHROMATOID RESIDUES FROM IMMATURE OR DEGENERATE ERYTHROCYTES IN GIEMSA-STAINED THIN FILMS, AND IN THICK FILMS STAINED BY THE ISOTONIC METHYLENE BLUE-AZURE-EOSIN METHOD DESCRIBED IN THE TEXT ( $\times 1700$ ).



# A NOTE ON THE RELATION BETWEEN THE VIRULENCE OF *TRYPANOSOMA RHODESIENSE* TOWARDS RATS AND THE NORMAL BLOOD TEMPERATURE OF ITS PREVIOUS MAMMALIAN HOST.

BY

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## INTRODUCTION.

The following data were obtained while the writer was in charge of the Human Trypanosomiasis Research Station at Tinde after the retirement of Dr. J. F. CORSON. The history of the *Trypanosoma rhodesiense* strain used there has already been published in detail (CORSON, 1939,\* etc.). Various antelopes and other animals infected with this strain of trypanosome were kept at Tinde (no other strains of species were maintained while the writer was in charge) and the strain was transmitted by *Glossina morsitans*. In order to isolate the infected flies, they were fed singly on numbered rats, which were examined for trypanosomes daily for a fortnight. Young healthy adult male rats were used during these isolations.

## OBSERVATIONS.

The observations obtained have been summarized in two tables. Table I gives the length of life in days of rats that were infected by *G. morsitans* transmitting *T. rhodesiense* from sheep, goat, monkey, eland, reedbuck, impalla, dik-dik, and Thomson's gazelle. The significance between the differences in the mean lives of the rats after infection is given in Table II.

Table II, column 1, gives the species of animal used, and column 2 gives the total number of infected animals used. Column 3 gives the mean observed anal blood temperature based on the corresponding number of individual animals given in column 4, and the total number of observations is given in column 5. Column 6 gives the percentage of *G. morsitans* which became infected after feeding on the corresponding infected host. This percentage is based on

\* CORSON, J. F. (1939). A fifth note on the infectivity to man of a strain of *Trypanosoma rhodesiense*; three further passages through antelopes and tests on man; two charts of the whole experiment. *J. trop. Med. Hyg.*, 42, 5.

TABLE I.

Length of Life in Days of Rats bitten by <i>G. morsitans</i> infected from :									
Sheep.	Goat.	Monkey.	Eland.	Reedbuck.			Impalla.	Dik-dik.	Thomson's gazelle.
50	48	98	62	41	34	33	30	17	36 35
56	56	44	65	40	61	30	46	25	33 33
66	54	55	41	45	28	33	41	30	45 34
62	53	52	40	35	72	32	28	18	49 36
70	56	51	66	60	46	28	35	46	31 19
64	60	54	63	50	48	40	33	41	48 28
65	66	58	65	36	60	41	48	42	46 36
73	64	64	50	50	36	42	43	50	40 18
90	50	62	54	33	50	43	30	35	40 14
104	68	48	31	30	50	30	28	37	35 31
51	77	82	28	56	66	36	33	20	25 28
48	72	84	42	22	52	35	37	28	43 22
68	65	70	33	61	56	42	39	30	28 29
71	81	50	37	51	62	22	46	23	34 33
65	86	54	35	40	61	33	38	42	29 21
74	62	53	36	62	40	30	36	39	22 40
46	66	38	40	41	48	36	47	45	30 38
73	59	45		35	63	38	48	43	38 19
76	68	47		29	48	40	47	46	35 20
64		52		40	56	29	49	41	21 18
				42	54			43	28 30
				35	62			35	18 28
				56	63			45	37 18
				57	70			36	33 16
				33	56			48	25 33
Mean	66.80	63.72	58.05	46.35	44.50		39.15	36.02	30.54

The significance between these differences is given in Table II.

the original numbers of flies fed upon the infected animal, and not upon the numbers of flies still living at the time of isolation. All the flies that died between the period of the first infective feed and time of isolation were dissected, and those with trypanosomes in the salivary glands were considered positive. The numbers of flies on which these percentages are based are given in column 7. Column 8 gives the mean length of life of the rats after the infective bite ; these means are taken from Table I. Column 9 gives the numbers of individuals on which the mean is based and column 10 the variance. Column 11 shows which differences are not significant; the significant value of P being taken as 1 in 100.

TABLE II.

1	2	3	4	5	6	7	8	9	10	11
Species (the source of trypanosomes)	Total number of infected animals used to transmit flies.	Rectal temperatures obtained before animals were infected.	Number of normal healthy animals used to obtain mean temperatures.	Total number of observations.	Percentage of original flies infected.	Total (original) numbers of <i>G. morsitans</i> used.	Mean length of life of rats infected by flies transmitting <i>T. rhodesiensis</i> from corresponding species of animal. See Table I for actual life in days.	Number of individual rats used for each species of animal.	Estimated variance of mean length of life.	Length of life <i>not</i> significantly different from that of :
Sheep ...	8	° F. 103.4	4	150	1.5	1,567	Days. 66.80	20	8.68	Goat
Goat ...	3	102.8	2	100	1.0	500	63.72	19	5.15	Sheep, monkey
Monkey ...	6	102.0	6	50	2.0	500	58.05	20	6.43	Goat
Eland ...	1	101.9	2	20	2.5	540	46.35	17	9.52	Reedbuck
Reedbuck ...	4	101.2	4	40	4.1	2,524	44.50	70	2.34	Eland
Impalla ...	2	100.8	2	20	4.5	522	39.15	20	2.22	Dik-dik
Dik-dik ...	4	99.6	2	20	6.0	600	36.02	25	5.03	Impalla
Thomson's gazelle	3	98.7	2	20	9.6	510	30.54	50	1.48	—

Correlation between columns 3 and 8,  $r = +0.6134$  (chances about 1 in 9).  
 " " " 3 and 6,  $r = -0.6135$  ( " " 1 in 9).



The percentage of flies infected (column 6) gives a negative correlation with the mean body temperature (column 3) with  $r = -0.6135$  (this is not significant, but the number of observations is small; the chances are 1 in 9). The mean longevity of the infected rats (column 8) also correlates positively with the mean body temperature (column 3) of the trypanosome's former host,  $r = +0.6134$  (not significant, but again, the number of observations is small and the chances are 1 in 9).

Some observations were also obtained with an ant-bear (*Orycteropus afer*) as original host. One individual was used and its mean body temperature was  $97.0^{\circ}$  F. from ten observations. Two per cent. of the *morsitans* became infective out of an original 400 flies fed on the animal for 4 days. Two rats, which were infected by one of these flies, lived 16 and 20 days respectively after the infective feed (mean longevity of 18 days).

#### CONCLUSION.

It appears that the transmissibility to the fly and the virulence of the strain of *T. rhodesiense* in rats may be enhanced by passing through animals with a low mean body temperature. This suggestion needs further investigation.

## CORRESPONDENCE.

### CRAZY PAVEMENT SKIN ERUPTION.

*To the Editor of the TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In East Africa skin changes apparently identical with the lesion described by Dr. LUCIUS NICHOLLS\* in these TRANSACTIONS are not uncommon in conditions far removed from those described by TROWELL in his article on infantile pellagra.†

During a survey of a native village school recently undertaken in Morogoro, Tanganyika, the incidence of this lesion was recorded as a possible sign of malnutrition. It was found only on the legs and was identical with that illustrated in Dr. NICHOLL's excellent photographs.

In the primary school, ages 6 to 13, among 240 boys the incidence was 19.2 per cent., while among 66 girls it was 33.5 per cent. In the secondary school among 262 boys 13.8 per cent. showed this sign. The children were divided into three groups according to an assessment of their nutrition based on general appearance and school behaviour only. Group I—those markedly thin and backward at games and in school; Group II—those who appeared reasonably well nourished and displayed average energy; Group III—those whose standard of physique and behaviour was above the average.

The distribution of the lesion is shown in the following table:—

	Group I.	Group II.	Group III.	Total.
Primary boys .. ..	34	167	39	240
Percentage with lesion	23.6	21.0	10.3	19.2
Primary girls .. ..	13	37	16	66
Percentage with lesion	46.2	40.5	6.2	33.5
Secondary boys .. ..	32	152	78	262
Percentage with lesion	31.2	12.5	8.9	13.8

The most striking feature is the much greater incidence of this sign among boys than girls; analysis showed this difference to have a very high significance.

\* NICHOLLS, L. (1940). Crazy pavement skin eruption. *Trans. R. Soc. trop. Med. Hyg.*, 34, 291.

† TROWELL, H. C. (1940). Infantile pellagra. *Ibid.*, 33, 389.

In the primary school the lesion is comparatively rare among the best nourished group and slightly more common in the poorest, but the difference in incidence between Groups I and II is without statistical significance. In the secondary school on the other hand the incidence is not significant between Groups II and III while it is markedly so between the poorest and the two better groups. The lower incidence among secondary as compared with the primary school boys has also a high degree of significance. An examination by age groups showed that the lesion was most common between the ages of 9 and 13, after which the incidence steadily decreased.

An analysis made to discover if any relationship existed between this and other signs of malnutrition, such as caries, spongy gums, low haemoglobin and parasitical infections, etc., gave no signs of any interdependence. In regard to follicular keratosis a highly significant antagonism was found; 3.1 per cent. only showing both lesions, while the incidence of follicular keratosis was 39 per cent., and of the cracked skin 18 per cent.

From these figures all that can be said as to the importance of this lesion is that it is *more common in girls than in boys* and *less frequent as age increases*. It is less common in children of good physique but in younger children the incidence is not markedly greater in obviously poorly nourished as compared with the average African child. It does not appear to be associated with any other sign of malnutrition and is rarely found in conjunction with signs of a lack of vitamin A.

I am, etc.,

ALAN MCKENZIE.

The Hospital,  
Chunya, Tanganyika.

TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL. XXXV. No. 2. SEPTEMBER, 1941.

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ANNUAL GENERAL MEETING

of the Society held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 17th July, 1941, at 4.30 p.m.

THE PRESIDENT

Sir S. RICKARD CHRISTOPHERS, C.I.E., F.R.S., Colonel I.M.S. (retd.),  
in the Chair.

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BUSINESS.

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REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1941.

The **Hon. Secretary**, Dr. WENYON, presented the Thirty-fourth Annual Report, copies of which were circulated at the meeting.

The thanks of the Council were expressed to those Fellows who had supported the Society whether by sending in their subscriptions regularly, nominating Candidates for Fellowship or contributing Papers suitable for publication in the TRANSACTIONS.

The adoption of the Report was proposed by Dr. STANNUS, seconded by Dr. STRAHAN, and the resolution was carried.

## REPORT OF THE HON. TREASURER FOR THE YEAR ENDED 31ST MARCH, 1941.

The **Hon. Treasurer**, Dr. O. MARRIOTT, presented his Report with the Accounts and Balance Sheet prepared by the Auditors, Messrs. W. B. Keen & Co., and approved by the Audit Committee.

Dr. MARRIOTT referred to the reduction in the Society's income from Fellows' subscriptions and from rents; and to various economies instituted to counter this. Some welcome donations had been received for the Manson House Fund, and in all the debt had been reduced by £591 during the year.

The adoption of the Report was proposed by Sir LEONARD ROGERS. The resolution was seconded by Dr. W. E. COOKE, and carried.

## ELECTION OF AUDIT COMMITTEE.

Sir MALCOLM WATSON proposed the re-election of the Audit Committee: Dr. V. S. HODSON, Colonel F. P. MACKIE and Dr. W. E. COOKE. This proposal was seconded and carried unanimously.

ELECTION OF PRESIDENT, VICE-PRESIDENTS, COUNCIL AND OFFICERS  
OF THE SOCIETY.

The **Hon. Secretary** announced that in view of war conditions, it was proposed by the Council to postpone the election of a new President, Vice-Presidents, Council and Officers of the Society, Sir RICKARD CHRISTOPHERS and other Officers of the Society having consented to remain in office for the time being. This was approved by the meeting.

This concluded the business of the Annual General Meeting.

TRANSACTIONS OF THE ROYAL SOCIETY OF  
TROPICAL MEDICINE AND HYGIENE.  
Vol. XXXV. No. 2. September, 1941.

## ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 17th July, 1941, at 4.45 p.m.

(After the Annual General Meeting.)

THE PRESIDENT,

Sir S. RICKARD CHRISTOPHERS, C.I.E., F.R.S., Colonel I.M.S. (retd.),  
in the Chair.

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## PAPER.

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### THE PRESENT POSITION OF YELLOW FEVER IN AFRICA

BY

G. M. FINDLAY, C.B.E., M.D., D.Sc.

*Wellcome Bureau of Scientific Research, London.*

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Just under one hundred years ago the President and Fellows of the Royal College of Physicians of London, in a report to the Lords of the Privy Council, stated that there was insufficient evidence to show that yellow fever was a disease *sui generis*. Today we know that yellow fever is a specific infection caused by a virus of approximately 22 m $\mu$  diameter.

Less than 25 years ago it was commonly believed that yellow fever was restricted in the Old World to a narrow strip of country on the West Coast of Africa, opinions differing, however, as to whether Sierra Leone, the Guinea Coast or the whole littoral must be regarded as the endemic home of the disease. Today we know that yellow fever extends from the southern borders

of the Sahara to the Belgian Congo, from the West Coast of Africa to the Anglo-Egyptian Sudan ; yet, despite increased knowledge, yellow fever in Africa still presents problems of great importance, the solution of which can only be obtained by the most careful practical application of the knowledge at present available : in addition, there are still lacunae which must be filled before it is possible to give a complete account of the epidemiology of that infection which was called by Sir GILBERT BLANE "the whirlwind of the human frame."

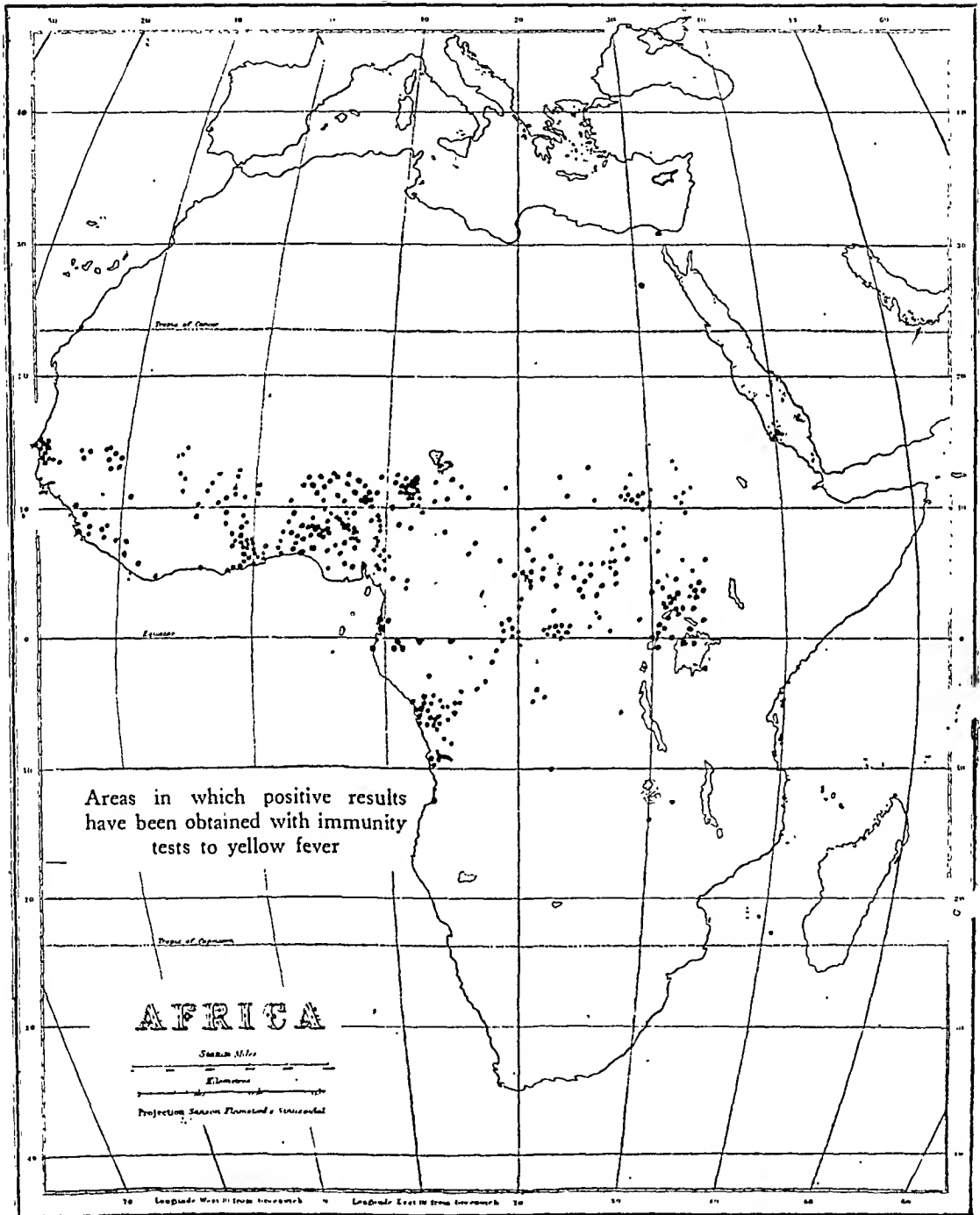
As the result of a recent journey to Africa, it has been possible to visit not only the British colonies on the West Coast but the Anglo-Egyptian Sudan, Kenya, Tanganyika Territory, Uganda, the Union of South Africa, Portuguese East Africa and the Chad Region : some experience has thus been gained of the difficulties which must be overcome if yellow fever in Africa is to be successfully controlled and its spread to India and the Far East effectively prevented. These difficulties are here briefly discussed and methods of overcoming them are suggested.

#### THE PRESENT DISTRIBUTION OF YELLOW FEVER IN AFRICA.

The distribution of yellow fever in Africa has in the past few years been accurately determined by immunity surveys involving the use of a mouse protection test by means of which the presence of immune bodies in the bloods of persons who have ever suffered from yellow fever may be readily demonstrated. When such surveys are carried out on different age groups of a population the period which has elapsed since the last extensive outbreak of yellow fever in a particular area may be accurately gauged. Histological examination of liver tissues from suspected cases has given evidence of when yellow fever is actually present.

Comparison of maps of Africa giving the distribution of yellow fever as determined by immunity surveys and by actual cases shows that the area in which positive results have been obtained by immunity surveys is only slightly more extensive than that in which cases of yellow fever have been demonstrated by histological examinations of livers. It is thus no longer possible to speak of "silent areas" in Africa while recent events in the Anglo-Egyptian Sudan have conclusively proved the specificity of the mouse protection test so far as the sera of human beings is concerned.

The area in Africa in which yellow fever is normally endemic thus involves most of the central part of the continent. Beginning on the West Coast of Senegal just north of Cape Verde, it follows the southern border of the Sahara to the Anglo-Egyptian Sudan ; then it bends southwards, involving the southern two-thirds of the Nuba Mountains in south-eastern Kordofan. It next crosses the White Nile south of Jebelein and passes through Dar Fung, the area between the White and Blue Nile, up to, and probably beyond, the Sudan-Abyssinian border. The eastern border of the area runs through the western part of





Uganda, keeping to the west of Lake Victoria and thence diagonally across the Belgian Congo to the mouth of the Congo River.

In order to determine whether any changes are occurring in this endemic area it would seem to be of importance to repeat surveys at intervals of 5 or 10 years, not only in the known endemic zone itself but in the border countries such as Kenya, Tanganyika, the Southern part of the Belgian Congo and Northern Rhodesia. The last extensive surveys in Nigeria, the Gold Coast and Sierra Leone were carried out 10 years ago, since when there have been considerable movements of population. The population of Freetown, for instance, has shown a very large increase in the past few years. The value of repeated surveys is illustrated by the case of Malakal in the Anglo-Egyptian Sudan. When the first survey was made in 1933 out of fifty sera, only one, obtained from a male aged 18, was found to be positive. A second survey in 1938 showed that nine out of fifty-two sera from children and twelve out of sixty-two sera from adults were now positive. The immunity rate had thus increased from 3.7 to 18.4 per cent. in the absence of any known epidemic of yellow fever or in fact of any case recognised at the time to be yellow fever.

Yellow fever has occurred in Africa not only in epidemics but even more strikingly in the form of isolated cases without any apparent relationship to the occurrence of epidemics. Thus in Freetown there had been no case of yellow fever since 1910 when, in 1935, a single European official died of the infection: isolated cases have more recently been reported from the Belgian Congo and from Accra. Efforts to find other cases in the neighbourhood have proved fruitless.

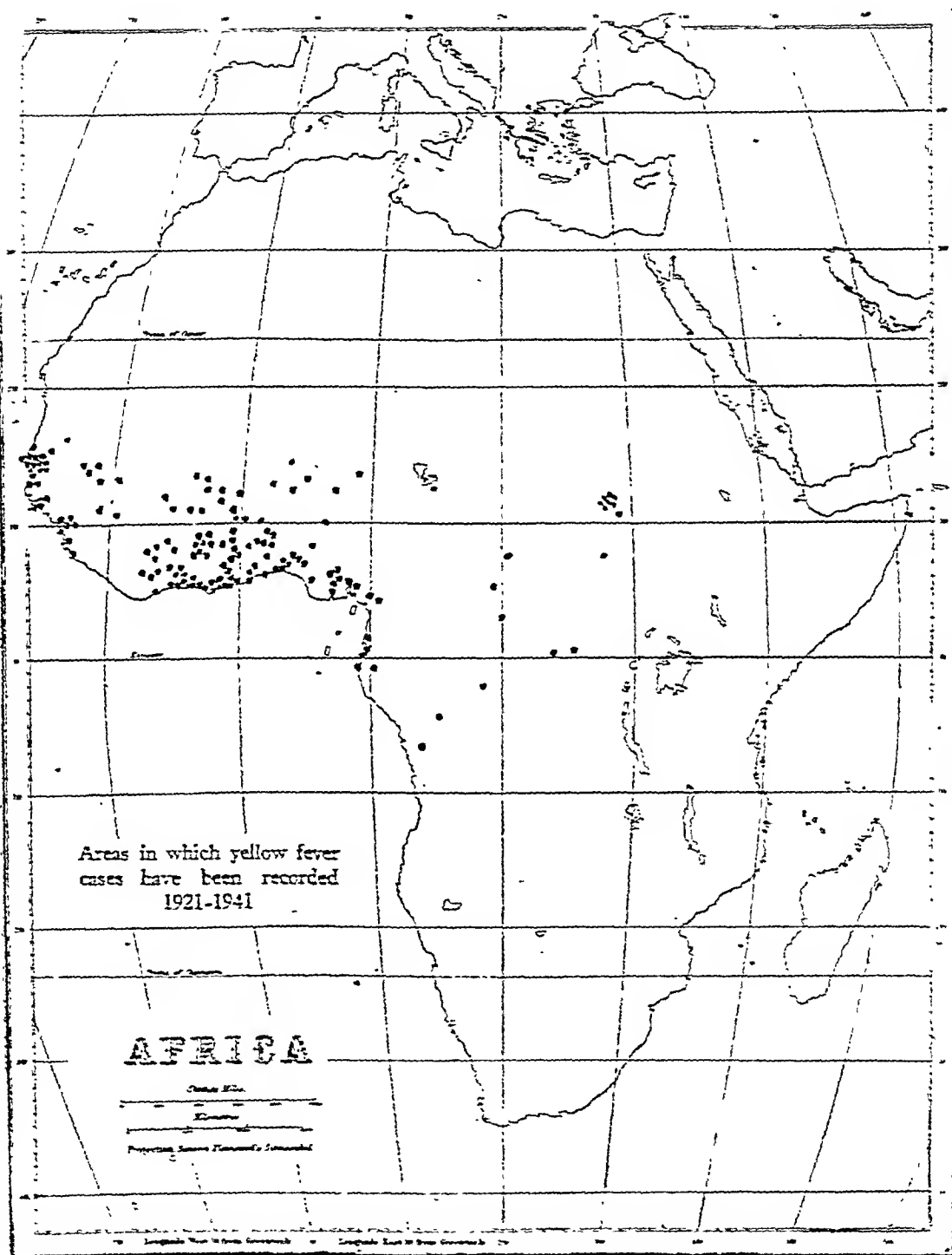
#### THE EPIDEMIOLOGY OF YELLOW FEVER.

It is possible to distinguish in Africa three epidemiological types of yellow fever:—

(1) Urban epidemicity. (2) Rural epidemicity. (3) Rural endemicity.

Typical urban outbreaks occurred in Lagos in 1925-26, in Bathurst in 1934-35, in Accra in 1926-27 and again in 1937. In all these urban epidemics there is no reason to incriminate as a vector any other mosquito than *Aedes aegypti* or to postulate in the continuance of the outbreak any other factors than infected mosquitoes and unimmunized men. Such epidemics are brought to an end either when infected mosquitoes have been destroyed or when a high percentage of the susceptible population has become immunized.

The most striking rural epidemic ever recorded in Africa is that which has recently occurred in the Anglo-Egyptian Sudan. An example on a far smaller scale was that in the Western Province of Ashanti on the Gold Coast in 1933, when in the isolated camp of a mining prospector three Europeans and a small number of Africans suffered from yellow fever. Another rural epidemic took place in 1937 on the Gold Coast in isolated villages in the Shai and Krobo



districts to the north and north-east of Accra. This rural outbreak was followed by a small explosive urban outbreak in Accra itself. In none of these rural epidemics were infected *A. aegypti* captured in association with actual yellow fever cases, but throughout Africa this mosquito is widely distributed and in certain areas may be found breeding not only in domestic and peridomestic situations but in tree holes, often at considerable distances from all human habitations. Throughout the Western Sudan ideal non-domestic breeding places are provided by hollow baobab (tebeldi) trees, *Adansonia digitata*. Many of these trees are naturally hollow, others have been artificially hollowed out, but in both cases the natural wells thus formed are used as cisterns for the storage of water during the dry season. The custom of employing baobab trees as natural cisterns is evidently one of great antiquity, for IBN BATTUTA, who visited the Sudan in 1353, draws attention to it. In certain regions during the dry season adult *A. aegypti* entirely disappear but their eggs may be found and bred out from the debris in tree holes. In some villages, however, where there is great scarcity or difficulty in obtaining water, *A. aegypti* may be found breeding at all seasons of the year in water pots kept in the huts.

In many villages the extent of the water supply is closely correlated with the breeding of *A. aegypti*. Where water is plentiful there is little tendency to store it in houses and hence little chance of mosquito breeding. The question whether *Taeniorhynchus* (*Mansonioides*) *africanus* acts as a vector in certain neighbourhoods as, for instance, round Malakal, requires further investigation. At present, however, it is impossible to exclude *A. aegypti* as a vector in rural epidemics of yellow fever in Africa, although other mosquitoes may play an accessory rôle. In epizootics of Rift Valley fever in Kenya the virus of that disease becomes so widely distributed in mosquitoes that it can be found in every species in the neighbourhood.

Rural endemicity is suggested as a convenient term to describe the occurrence of isolated cases as in the Belgian Congo in 1937 and 1940, and in the French Sudan in 1938, together with the finding in many small rural communities in Nigeria, the Gold Coast, the Gambia, the Sudan, Uganda and the Belgian Congo of low rates of immunity in the human population in the absence of any known epidemic of yellow fever. Since in many of these areas all age groups show some degree of immunity to yellow fever, it is probable that infection has occurred again and again in the same community. How this rural endemic infection is carried on is unknown. There are, however, certain suggestive possibilities which require consideration.

Endemic yellow fever might be carried on solely by the mosquito-man cycle through the continued presence of infected mosquitoes and the frequent occurrence of human cases. If cases were occurring continuously in man in any particular area it should be possible to obtain virus from the blood of these patients for, even if the vast majority had mild or even subclinical symptoms, a few more or less typical cases should be seen from time to time. Nevertheless,

efforts to isolate the virus from patients with febrile symptoms in areas where mouse protection tests show a low rate of endemicity have not been successful.

A second possibility exists: the virus may be carried on by a mosquito-animal cycle, animals other than man being infected by the bites of infected mosquitoes and in their turn serving to infect other mosquitoes. There is considerable evidence to show that hosts other than man exist among wild and domestic animals. Attention was first drawn by FINDLAY *et al.* (1936) to the presence of immune bodies in the blood of a monkey *Procolobus badius waldroni* from the Gold Coast. Since then examinations have been made of the sera from 123 monkeys and apes caught in areas where yellow fever is endemic in man (cf. Table I): 24 sera or 19.5 per cent. have given positive results. The sera of monkeys from East Africa and from India have given negative results. There is therefore no reason to doubt the specificity of the mouse protection test when applied to the blood of primates.

A small number of sera from wild animals—chiefly bats, squirrels and other small rodents—have also been examined for protective antibodies against yellow fever. These have all proved negative, but before it is possible to give an answer to the question whether such animals play any rôle in maintaining endemic

TABLE I.  
IMMUNE BODIES IN THE BLOODS OF AFRICAN PRIMATES.

Country.	Species.	Number of Sera Tested.	Number of Sera Positive.	Observers.
GAMBIA ...	<i>Mandrillus sphinx</i> ...	1	0	FINDLAY <i>et al.</i> (1936)
" ...	<i>Cercopithecus aethiops</i>	4	0	"
GOLD COAST ...	<i>Procolobus badius waldroni</i>	1	1	"
	<i>P. badius waldroni</i>	3	1	FINDLAY & MACCALLUM (1937)
	<i>Erythrocebus patas</i>	1	0	FINDLAY <i>et al.</i> (1936)
	<i>E. patas</i>			FINDLAY & MACCALLUM (1937)
	<i>Cercopithecus diana rolozway</i>	1	0	FINDLAY <i>et al.</i> (1936)
	<i>C. diana rolozway</i>	1	1	FINDLAY & MACCALLUM (1937)
	<i>Cercopithecus species</i>	1	0	FINDLAY & MACCALLUM (1937)
	<i>C. mona lorwei</i>	1	0	FINDLAY <i>et al.</i> (1936)
	<i>Colobus vellerosus</i>	5	3	FINDLAY & MACCALLUM (1937)
	<i>Procolobus badius badius</i>	6	3	FINDLAY & MACCALLUM (1937)

TABLE I—(continued).

Country.	Species.	Number of Sera Tested.	Number of Sera Positive.	Observers.
LIBERIA ...	<i>Cercopithecus species</i>	10	4	FINDLAY & MACCALLUM (1937).
SIERRA LEONE	<i>Colobus vellerosus</i>	1	1	FINDLAY & MACCALLUM (1937).
UGANDA	<i>Cercopithecus aethiops centralis</i>	20	5	FINDLAY & MACCALLUM (1937).
ANGLO-EGYPTIAN SUDAN	<i>C. aethiops centralis</i>	15	1	FINDLAY & MACCALLUM (1937)
	<i>C. aethiops centralis</i>	3	1	FINDLAY <i>et al.</i> (1938)
	<i>Erythrocebus patas</i>	1	0	FINDLAY & MACCALLUM (1937)
	<i>E. patas</i>	2	0	FINDLAY & MACCALLUM (1937)
	<i>Galago senegalensis</i>	3	0	FINDLAY & MACCALLUM (1937)
	<i>senegalensis</i>	3	0	(1937)
	<i>Galago senegalensis senegalensis</i>	7	0	FINDLAY <i>et al.</i> (1938)
BELGIAN CONGO	<i>Papio species</i>	1	1	FINDLAY <i>et al.</i> (1936)
	<i>Allenopithecus nigroviridis</i>	1	0	FINDLAY <i>et al.</i> (1936)
	<i>Cercopithecus cephus</i>	2	0	FINDLAY <i>et al.</i> (1936)
	<i>C. neglectus</i>	1	0	FINDLAY <i>et al.</i> (1936)
	<i>C. aethiops cynosurus</i>	1	0	FINDLAY <i>et al.</i> (1936)
	<i>Cercocebus galeritus agilis</i>	4	0	FINDLAY <i>et al.</i> (1936)
	<i>C. galeritus agilis</i>	1	0	VAN DEN BERGHE (1939)
	<i>Colobus vellerosus</i>	1	1	VAN DEN BERGHE (1939)
	<i>Papio anubis</i>	2	0	VAN DEN BERGHE (1939)
	<i>Papio jubilaus</i>	2	0	VAN DEN BERGHE (1939)
	<i>Pan satyrus schweinfurthi</i>	5	0	VAN DEN BERGHE (1939)
FRENCH GUINEA	<i>Pan satyrus schweinfurthi</i>	6	1	FINDLAY <i>et al.</i> (1936)
FRENCH EQUATORIAL AFRICA	<i>Gorilla gorilla</i>	2	0	SALEUN (1938)
	<i>Pan satyrus schweinfurthi</i>	3	0	SALEUN (1938)
	<i>Pan satyrus schweinfurthi</i>	1	0	SALEUN (1938)
	<i>Papio species</i>	1	0	SALEUN (1938)
	<i>Cercopithecus species</i>	1	0	SALEUN (1938)
	<i>Cercopithecus aethiops cynosurus</i>	1	0	SALEUN (1938)

yellow fever it will be necessary to examine some hundreds of specimens, instead of as at present just under fifty. Such an investigation is of importance in view of the fact that, in Colombia, BUGHER (1940) has found that specimens of the domestic pig (*Sus*) and the peccary (*Tayassu*) may show immune bodies

TABLE II.

MOUSE PROTECTION TESTS AGAINST YELLOW FEVER VIRUS IN THE BLOODS OF DOMESTIC ANIMALS AND BIRDS.

Species.	Country.	Number of Sera Tested.	Number of Sera Positive.	Percentage Positive.	Observers.
Cow ...	Anglo - Egyptian Sudan	50	7	14	MACCALLUM & FINDLAY (1937) FINDLAY <i>et al.</i> (1938)
	Sierra Leone ...	16	1	6.2	MACCALLUM & FINDLAY (1937)
	Gold Coast ...	15	0	0	MACCALLUM & FINDLAY (1937)
	Uganda ...	22	12	54.5	MACCALLUM & FINDLAY (1937)
	French Equatorial Africa	13	5	38.4	SALEUN (1939)
Sheep and Goats	Gambia ...	8	2	25	FINDLAY <i>et al.</i> (1938).
	Anglo - Egyptian Sudan	7	0	0	FINDLAY <i>et al.</i> (1938)
	Nigeria ...	100	32	32	SMITH (1940).
	French Equatorial Africa	6	3	50	SALEUN (1939)
Horses ...	French Equatorial Africa	6	3	50	SALEUN (1939)
Pigs ...	Anglo - Egyptian Sudan	11	3	27.2	FINDLAY <i>et al.</i> (1938)
Camels ...	French Equatorial Africa	5	3	60.0	SALEUN (1939)
Dogs ...	Anglo - Egyptian Sudan	5	2	40.0	FINDLAY <i>et al.</i> (1938)
	French Equatorial Africa	4	1	25.0	SALEUN (1939).
Ostriches	French Equatorial Africa	2	1	50.0	SALEUN (1939)
Hens ...	Gambia ...	14	0	0	FINDLAY <i>et al.</i> (1936)
	Anglo - Egyptian Sudan	6	0	0	FINDLAY <i>et al.</i> (1938)

to yellow fever in their bloods while among birds the buzzards, *Cathartes* and *Coragyps*, showed some very slight degree of antiviral activity. In a more recent communication, BUGHER and his colleagues (1941) have shown that marsupials such as opossums may play a part in Colombia in maintaining endemic yellow fever virus: of 265 sera tested, 18 or 6·7 per cent. showed immunity to yellow fever.

Among domestic animals from yellow fever endemic areas in Africa a considerable percentage, however, have shown the presence of virucidal bodies in their blood. Thus of 166 cows, 25 or 21·5 per cent. have been shown positive, of 121 sheep and goats 27 or 30·5 per cent., of six horses 3 or 50 per cent., of eleven pigs 3 or 27·2 per cent., of five camels 3 or 60 per cent., of nine dogs 3 or 33·3 per cent., of two ostriches 1 or 50 per cent., and of twenty hens, 0 (cf. Table II). As, however, virucidal bodies have been found in the blood of sheep and cattle (MACCALLUM and FINDLAY, 1937), from areas where yellow fever is not known to be endemic although always in lower concentration, the full significance of these findings in domestic animals in Africa must await further investigations.

It is possible, however, that yellow fever endemicity may be maintained by preservation of the virus for long periods in some vertebrate or possibly non-vertebrate host. It must be remembered that although a mosquito once infected remains so for life its average life span probably does not exceed 2 months: in all vertebrates so far tested the virus persists in the peripheral blood stream only for a few days. Now in certain areas where yellow fever appears to be endemic there exists a prolonged dry season during which mosquitoes are extremely scarce even if not actually non-existent. It is thus not easy to see how infection can be maintained for long periods solely by the mosquito-man or mosquito-animal cycle unless there is hereditary transmission through the mosquito egg. This does not occur in *A. aegypti* but it may possibly occur with some other insect vector.

It may be necessary, however, to postulate the presence of a reservoir of the virus from which on occasions the mosquito-man or mosquito-animal cycle may be again set up. In this connection it is interesting to note that African horse sickness, a virus infection with certain similarities to yellow fever has been found to remain latent in an area for as long as 15 years in the absence of all Equidae.

No direct evidence that yellow fever can be carried for long periods by any invertebrates other than mosquitoes has been found. However, the possibility cannot be entirely dismissed for in the cockroach the viscerotropic virus can persist up to 15 days (FINDLAY & MACCALLUM, 1939a), while as unpublished observations show, it can persist in the leech up to 8 days. The survival of the virus in certain invertebrates is shown in Table III.

TABLE III.

Species.				Length of Survival in Days.
<i>Blatta orientalis</i> —Cockroach	..	..	..	Less than 2
<i>Blatta germanica</i> —Cockroach	..	..	..	15
<i>Schistocerca gregaria</i> —Desert locust	..	..		Less than 2
<i>Locusta migratoria migratoria</i> —African migratory locust	..	..	..	Less than 2
<i>Hirudo medicinalis</i> —Leech	..	..	..	8
<i>Agriolimax agrestis</i> —Slug	..	..	..	Less than 2
<i>Glossina morsitans</i> —Tsetse fly	..	..	..	Less than 2

That mosquito bites are not the only known means of transmission of yellow fever has been realized by the ease with which those who are not immunized become infected when working with the virus under laboratory conditions. FINDLAY & MACCALLUM (1939b) have drawn attention to the fact that rhesus monkeys may develop spontaneous infection if they are confined in a room which has contained infected monkeys some months previously. In other words, the infection can persist in the absence of infected mosquitoes.

It is obvious that further research alone can fill in the lacunae in our present knowledge of the epidemiology of yellow fever.

#### THE POSSIBLE SPREAD OF YELLOW FEVER.

From what has been said of the possible rôle of animals in maintaining endemic infection, it follows that theoretically yellow fever might be transferred from one area to another by migratory animals or birds. At present, however, the only two definite factors by which spread is visualized as occurring is by the infected mosquito or the infected human being.

*The infected mosquito.* Unlike some ædine mosquitoes, *A. aegypti* does not normally fly for long distances but tends rather to haunt the area in which it was bred; nevertheless, on some occasions and with the aid of a following wind marked specimens have been recovered at a considerable distance from their point of liberation. WISEMAN, SYMES, MCMAHON and TEESDALE (1939) found that when the south-west monsoon was blowing *A. aegypti* was able to cross the 600 to 800 yards of water separating Mombasa Island from the mainland at Likona. Observations on the range of flight of other potential mosquito vectors of yellow fever are meagre. *Culex fatigans*, however, was found by AFRIDI and ABDUL MAJID (1932) to travel 5,500 yards in India, while *Culex thalassius* has been caught at Hill Station, Freetown, some 2 miles from its breeding ground (DAVEY, 1941).

On the other hand, since a mosquito once infected remains infected for life, there is time for it to be transported passively for considerable distances by air, rail, road or ship.

*The infected human being.* After the bite of a mosquito infected with yellow fever, the incubation period of the disease in man varies from 2 to 6 days.



During the latter part of this incubation period and during the first three days of fever the virus may be present in the circulating blood, the time during which the individual is infective to mosquitoes thus varying from 4 to 7 days. With modern methods of transport there is thus ample time for anyone to pass from an endemic to a non-endemic area during the period before or after he becomes infective.

Before reviewing very briefly the possibilities of spread of yellow fever, it is pertinent to ask whether yellow fever has been known to spread in historic time. During the seventeenth, eighteenth and nineteenth centuries there were numerous occasions on which yellow fever was transported by boats. The introduction of yellow fever into Martinique in 1686 by the French warship, the *Oriflamme*, which had called at a Brazilian port is well known, as is the transport of the disease in 1823 from Sierra Leone to Ascension Island by H.M.S. *Bann*, and the carriage of infection from Sierra Leone to Boà Vista, one of the Cape Verde Islands, by H.M.S. *Eclair* in 1845. In 1865 the *Hecla*, from Cuba, introduced the disease into Swansea so that from 15th September to 4th October twenty persons in the town in definite relation to the ship were attacked, together with the crew of a small vessel that had been lying alongside the *Hecla*. In Africa itself urban outbreaks such as that in Accra in 1937 have been due to the arrival in the town of infected individuals. Whether yellow fever moved from West Africa to the Sudan, or vice-versa or whether it has been endemic throughout its present area for countless generations will probably never be known. A rather better case, however, can be made out on general grounds for a spread from east to west rather than from west to east.

The means of spread comprise aeroplanes, railways, boats and motor-cars.

### (1) *Aeroplanes.*

The principal air routes now operating in Africa in relation to yellow fever areas, are those under the combined control of British Overseas Airways Corporation, South African Airways and the Belgian Sabena Airways. Before the war Air France and the Deutsch Luft Hansa Companies touched at Dakar and Bathurst on their way to Brazil. The main north to south route of British Overseas Airways runs from Alexandria to Durban. At Alexandria connection is made with planes going to India, Australia and the Far East.

The north to south air route passes through two countries in which yellow fever is endemic, the Anglo-Egyptian Sudan and Uganda. In the Sudan south of Khartoum stops are made at Malakal and at Rejaf: in Uganda at Port Bell (Kampala). Natives at Malakal and Rejaf are known to have immune bodies to yellow fever in their bloods but at Kampala no evidence of infection has been found. From Port Bell the air route runs through Kenya, with stops at Kisumu and Mombasa, thence to Dar-es-Salaam, Mozambique, Beira, the Vaal Dam and Durban. From Khartoum another air route passes south to Juba, thence through the Belgian Congo to Duala and Lagos. The more direct route from Khartoum to Lagos passes westwards, with night stops at either Fasher or Geneina, both of which are in the endemic zone, Fort Lamy in

French Chad territory, to Maiduguri, Kano, Kaduna, Ogbomosho and Lagos and thence to Takoradi on the Gold Coast.

South African Air Lines operate a route from Germiston (Johannesburg) through Bulawayo, Lusaka, Ndola, Elisabethville, Kasama, Dodoma, Nairobi, Kisumu, Entebbe, Irumu, Coquilhatville and Léopoldville, as well as a line from Germiston to Loanda in Angola.

A seaplane service also operates down the West Coast with stops at Bathurst, Freetown and Lagos.

With the daily flights, often of more than 1,000 miles, now made by commercial aircraft, it is obvious that yellow fever might be distributed far and wide.

Although the carriage of *A. aegypti* by plane may appear a remote possibility, this mosquito is occasionally found in planes, one having been captured in the past few months at Mombasa in a plane arriving from Dar-es-Salaam.

The measures taken to prevent mosquitoes being carried by plane are (1) fitting, (2) the provision of anti-amaril aerodromes. At present fitting is carried out infrequently and in some cases inefficiently. On two flights between Khartoum and Lagos no fitting was done except at Kano and then only some considerable time after the plane had landed and the doors had been opened. On the Khartoum-Durban route fitting was carried out, very efficiently at Kisumu, on the West Coast route only at Freetown. Insufficient precautions are taken to ensure that passengers do not become infected at night stops. At Geneina, at present used as a night stop, there are unscreened huts for passengers and Africans in close proximity to the landing ground despite the fact that immune bodies to yellow fever have been found in the bloods of the local inhabitants. Seaplanes are often moored at distances of less than 600 to 800 yards from the shore. Even if closed at night they are inevitably opened up in the early hours of the morning and evening when *A. aegypti* is most likely to be active.

If the measures taken to prevent the entry of mosquitoes into planes are inefficient those at present taken to prevent the entry of a person who is or may become infective are ineffective. Some countries before allowing entry are content to accept a certificate from an unimmunized person coming from a known endemic zone to the effect that he has not been exposed to infection with yellow fever for the 6 days prior to embarkation. Unless the 6 days have been passed in a mosquito-proof dwelling it is impossible for any person to assert that he may not have been exposed to infection. If a person has been immunized against yellow fever certain countries refuse him admittance unless 10, 21 or 23 days have elapsed since the date of his inoculation. Since there is now agreement that a person who has been successfully vaccinated against yellow fever is immune 10 days after inoculation there is no scientific basis for the imposition of quarantine periods of 21 days and upwards. In inoculating persons against yellow fever who are already in the tropics great care should

be taken to see that the vaccine employed is active, for as it is thermolabile it may easily be rendered inert.

### (2) Railways.

As in the case of aeroplanes, the possibility of carrying infected mosquitoes or infected passengers exists in the case of railways. Unfortunately, railway stations and their environs are often a prolific breeding ground for *A. aegypti* owing to the presence of water tanks. Luckily, however, the possibility of introducing yellow fever from an endemic to a non-endemic area by rail is fairly remote since there is no instance of an infected and a non-infected area being directly connected by rail, Kampala, the western terminus of the Uganda-Kenya railways being to the east of the endemic zone in Uganda. The Tanganyika railway, however, connects with steamers on Lake Victoria, whence it is possible to reach Uganda and through Kigoma and Lake Tanganyika the Belgian Congo. In the Anglo-Egyptian Sudan the whole of the railway system is north of the endemic zone. Nevertheless, it would be possible for an infected person from Kordofan or from further west, Darfur or even Chad Territory, to join the train at El Obeid and proceed, as is done during the pilgrimage to Mecca, to Suakin or Port Sudan on the Red Sea coast. Both of these ports are known to be infected with *A. aegypti*.

In the British West African colonies only the question of internal spread arises. Whether infection has been carried by rail to Accra, or Lagos from the interior appears to be unknown.

### (3) Boats.

The possibility that spread may still take place by boat must be kept in mind. Even as late as 1926 cases occurred on board ship in Lagos harbour. On the east coast of Africa there is, of course, little danger from ships unless yellow fever actually occurs in one of the ports. There does, however, exist a possibility that infection may be transmitted to India by an infected person going on board a ship carrying *A. aegypti*. Every fortnight some 400 Asiatics, half of them from Uganda, leave Mombasa for India. Although the port of Mombasa is now free from mosquitoes, Durban, Beira and Lorenço Marques are not, and the possibility of *A. aegypti* getting on board ship at these ports is considerable. If an Indian were to become infected in Uganda it would be quite possible for him to board a ship in Mombasa either before or during the period when virus is circulating in his blood, and thus to infect any mosquitoes which were lurking on the boat. The extrinsic incubation period in the mosquito would just be over when the ship after a voyage of 10 to 12 days arrived at an Indian port. In view of this possibility it would not be unreasonable if the Government of India were to insist that all persons leaving Mombasa for India should be immunized against yellow fever.

On the East Coast of Africa a large dhow traffic exists, partly coastal, partly transoceanic. The coastal dhow traffic extends from Mogadishu on the north to Dar-es-Salaam on the south. The transoceanic traffic is chiefly between

Africa and Arabia : in the Red Sea between Port Sudan and the Arabian ports of Jeddah, Wej and Yembo ; in the Indian Ocean between the south coast of Arabia and Mombasa and Dar-es-Salaam. Recently, however, large ocean-going dhows of approximately 150 tons are making direct sailings from Bombay to the African Coast. Unless the East African coast is actually infected with yellow fever dhow traffic is not likely to become a factor in the spread of the disease. Nevertheless, many dhows carry large unscreened water tanks in which *A. aegypti* breeds freely while at certain times of the year rain-water may collect in the bilges forming additional breeding foci. Dhows infested with *A. aegypti* may thus serve to reinfect coastal towns, an event which is said to have occurred in Port Sudan from dhows anchored in Flamingo Bay. At present strenuous efforts are being made both in Mombasa and in Dar-es-Salaam to provide dhows with mosquito-proof water tanks.

Boats on inland waterways in Africa are unlikely to harbour mosquitoes since there is no reason to store fresh water on board. There is, however, the possibility that they may carry persons during the infectious stage. Thus it may be recalled that on the White Nile the towns of Rejaf, Juba, Malakal, Tonga and Kaka are known to have been infected with yellow fever, while Jebelein and those further down stream have not. Boats on Lakes Victoria and Tanganyika might conceivably help to transmit infected persons from Uganda or the Belgian Congo to Kenya or Tanganyika.

#### (4) *Motor Cars.*

The transmission of *A. aegypti* in closed cars is probably a somewhat remote possibility but the carriage of infected persons over considerable distances by car is perhaps the most likely means by which yellow fever may spread in Africa.

During the past few years there has been in Africa a large increase both in the number and mileage of all-weather motor roads. It is now easily possible to motor long distances in a short time as, for instance from Stanleyville to Nairobi in 4 days or to Juba from a point in the Belgian Congo, some 300 km. away, in a single day, instances that have actually occurred in the past few months. Many new roads have recently been constructed as a result of military exigencies. These new roads are being constantly used not only by what may be called " through traffic " but by persons taking holiday tours. Such individuals often have no fixed itinerary but camp as the spirit moves them at night in rest houses or simply by the roadside. In addition to Europeans, Syrians and Indians, more and more Africans are now using the roads in ever-increasing number since with the advent of motor 'buses, long-distance travel has become financially within the reach of all. Finally, yellow fever may be carried by persons walking from one village to another. The fact that during the wet season so little motor traffic has been on the roads is possibly one of the factors that have militated in the past against the spread of the disease.

Many factors thus suggest that the spread of yellow fever to fresh countries is by no means a remote possibility. Two objections to this view have, however, been urged. Thus it has been suggested that some biological barrier prevents the extension of yellow fever to East and South Africa. The nature of this biological barrier is usually unspecified, but the possibility that some races of *A. aegypti* are unable to transmit the virus has been postulated. Observations on strains of this mosquito from Tanganyika Territory, India and the Far East show that all are equally capable of transmitting the virus by bite.

A second objection to the possible spread of yellow fever is that if it were likely to spread it should have spread years ago. Up to the present the barriers to the spread of infection would seem to have been sociological and geographical. Where man can only proceed on foot or on animals, deserts, mountains and marshes present a formidable hindrance to rapid communication and hence to the spread of disease. The Pibor Marshes, for instance, which lie to the south of the Fung area in the Sudan, have probably prevented infection from spreading further south to the Tapotha and Latuka tribes. Today geographical barriers have been almost entirely overcome by the internal-combustion engine.

A few years ago native movement was limited by internicine war so that any African who strayed too far from his own tribal area was only too liable to death or enslavement. Today such hindrances to movement have largely disappeared.

That the spread of disease owing to increased rapidity of transport is not wholly illusory is shown by the sudden appearance in Brazil of *Anopheles gambiae*, with the subsequent occurrence of thousands of fresh cases of malaria. Although the West Coast of Africa has been closely connected with South America for many centuries it is only in the last six years that *A. gambiae* has crossed the South Atlantic from Dakar to Natal (Brazil) as a result of the use of fast torpedo boats in association with the air services to South America. It is also reported on reliable authority that a Panama mosquito, *Deinocerites spanius*, which fortunately does not bite man, has been found in the United States of America near Brownsville, Texas. It has, apparently, been carried from Central America by plane. *Anopheles darlingi*, a dangerous vector of malaria, has in addition recently been found in British Honduras far to the north of its normal South American home.

#### THE CONTROL OF YELLOW FEVER.

From what has been said of the epidemiology of yellow fever it is obvious that eradication of the disease from West Africa is as yet impossible and will continue to be impossible so long as the factors underlying the endemicity of the disease remain unsolved. Nevertheless much may be done to eliminate epidemics from the present infected zone and to limit the spread of the disease to areas where the infection is now unknown.

The methods of prevention include: (1) Accurate and early diagnosis; (2) Destruction of mosquitoes; (3) Immunization; (4) Administrative measures.

(1) *Accurate and Early Diagnosis.*

If an epidemic is to be controlled in its early stages it is essential that it should be rapidly and accurately diagnosed. Unfortunately there is no one definite clinical sign by which it is possible to diagnose yellow fever. Severe cases may be confused with leptospiral jaundice, relapsing fever, "Kukuruku" disease, pneumonia, infective hepatitis, acute and subacute necrosis of the liver and malaria, while mild cases may be confused with influenza. It follows, therefore, that no diagnosis of yellow fever made on clinical grounds alone should henceforth be accepted. The only certain diagnosis of yellow fever should be based on:—

- (a) Examination of liver tissues in fatal cases by a competent pathologist.
- (b) Mouse protection tests carried out on the serum of the patient during the first few days of fever and again during convalescence.
- (c) Isolation of virus from the blood.

In the liver the changes characteristic of yellow fever should be found microscopically while the mouse protection tests should show a significant increase in immune bodies during the course of the disease.

Isolation of the virus from the blood can only be carried out during the first three days of fever by intracerebral injection of blood into mice or intraperitoneal injection into rhesus monkeys, or hedgehogs.

It is doubtful whether in view of the religious beliefs and primitive character of the tribes in many parts of Africa the time is yet ripe for a viscerotomy service on the lines of that employed in South America. It is urged, however, that every medical officer serving in a British possession in Africa should be furnished with and should know how to use a viscerotome. Whenever a full postmortem is unobtainable in patients who have died from an illness lasting less than 10 days every effort should be made to obtain a liver specimen by viscerotomy. Blood for immunity tests should be taken early and late in the disease from all cases of pyrexia of unknown origin, while during the first few days of fever blood should be inoculated into susceptible animals.

Although in certain parts of West Africa the inhabitants are sophisticated enough to regard all cases of jaundice as yellow fever there are many areas in Nigeria, the Sudan and elsewhere which are so isolated, especially during the rains, as to take from four to six weeks to reach by ordinary means of transport. In these areas epidemics of yellow fever, malaria and relapsing fever have undoubtedly occurred without anyone in authority being aware of them.

In these circumstances political officers could do much by urging on chiefs and other notables the importance of at once notifying any undue mortality

in the areas under their control. More might also be done to impress on native administrations the dangers to themselves and others of the spread of epidemic diseases.

In view of the great difficulties in reaching the sites of epidemics by road or rail and the impossible delays often entailed in getting important pathological material to laboratories where alone accurate diagnosis can be made, the use of light aeroplanes should be seriously considered in countries such as Nigeria, the Sudan and Tanganyika Territory.

## (2) Mosquito Destruction.

An essential preliminary to the destruction of mosquitoes is an adequate survey of the breeding both of *A. aegypti* and other potential yellow fever vectors, together with the provision of an accurate *Aedes* index not only for house breeding but for tree hole and outside breeding (tins, cocoa-nut shells, snail shells, etc). All mosquito surveys, to be of value, should be made under the supervision of a European medical entomologist and the identification of mosquitoes should not be left to partially trained Africans. In order to limit the breeding of mosquitoes in houses and compounds the provision of an adequate water supply is essential. Where supplies are inadequate water is inevitably stored in pots in or near houses and mosquitoes inevitably enter houses.

In smaller towns the provision of screened wells would meet the case: in larger cities such as Ibadan, the provision of an adequate piped water supply for the whole town would not only reduce mosquito breeding but would limit the use of streams heavily contaminated with the faeces of animals and man as sources of drinking water. Another important factor in reducing mosquito breeding is the elimination of over-crowding. Clearance of over-crowded areas is being gradually carried out in Lagos but in Bathurst the population massed on the Island of St. Mary still lives in one of the worst slums in tropical Africa. In some towns there is considerable opposition to the removal of gutters from houses; other towns are able to dispense with all gutters. If gutters are retained short lengths only should be provided above doorways and holes should be bored at intervals so that water cannot stagnate and form breeding places for mosquitoes. Standing water is often left for long periods in fetish or juju houses, mosques and churches: flower vases in Christian cemeteries are also liable to breed mosquitoes; all these should be examined periodically for breeding.

In some urban areas careful consideration is given to the question of mosquito breeding in tree holes, in others little or no attention is paid to this point. It should be emphasized that a common preliminary to the formation of rot holes in trees is unskilled lopping. Where trees have numerous holes they should be felled. When holes are to be filled up they should first

be creosoted and then filled with a mixture of coals, or other tar preparation, and sand. This does not crack in the same way as cement.

While the elimination of *aëdine* mosquitoes from rural areas in Africa is at present out of the question more might be done to destroy these mosquitoes in urban areas both in West and East Africa. The essential steps for the elimination of *aëdes* are known; it is a question of the supply of men and money. Whatever steps are taken it is of the greatest importance that they should be maintained and not allowed, as has happened in the past, to lapse after a few years.

As a means of reducing the chances of infection from the bites of mosquitoes the principle of segregating European residences in West Africa has for many years been approved by the Colonial Office. Despite this official approval and numerous pronouncements by Colonial Governments segregation is still not insisted upon in West Africa. European residences are built either in close proximity to African habitations or African servants, their wives and children are permitted to reside in the compounds of Europeans. In addition, rest houses and particularly mission stations are often built too close to African villages.

It has been urged that successful immunization against yellow fever will reduce or entirely remove the necessity for European segregation. This will be true only if and when immunization is made compulsory and is rigidly enforced not only for Government officials but for traders, missionaries and other non-Africans. Even then the presence of African servants and their children would constitute a great danger to Europeans since the chances of infection with malaria and dengue still persist. That no great inconvenience is caused to Europeans or their servants by prohibiting the latter from living in European compounds is shown by the example of Freetown, where this prohibition has been in force for some years.

### (3) *Immunization*

The discovery of a safe and effective means of immunization constitutes the most important recent advance in the control of yellow fever. Unfortunately the vaccine is a living virus and is rapidly destroyed if exposed to the high temperatures which may be encountered in the tropics. The vaccine should, therefore, be kept continuously at a temperature of not more than 0° C: temperatures below freezing point are, however, even more satisfactory. In this connection it is important to remember that when placed in a refrigerator the vaccine should be kept in the coil and not in the body of the machine. With the Frigidaire control set at number 1 the temperature of the coil is approximately -4° C. but of the body anything from +10° to +15° C. When transported outside a laboratory the vaccine must be kept in thermos flasks tightly packed with a mixture of ice and salts (2 parts to 1 part). No vaccine after its arrival in Africa should be used before it has been tested out in mice



and this testing should be repeated at intervals of from six to eight weeks. This would entail the keeping of mice in laboratories throughout Africa but as mice are now essential for the diagnosis of many virus infections such an innovation would be of use not only in relation to yellow fever.

For some years past, in fact since 1932, facilities have been available in London at the Wellcome Bureau of Scientific Research for the immunization of Government officials, missionaries, traders and others proceeding to West Africa and other endemic centres of yellow fever. Many thousands have now been inoculated and a considerable percentage of Europeans residing in British West Africa are now immunized against yellow fever. More recently immunization has been extended to the Anglo-Egyptian Sudan and Uganda.

For some years the Government of the Gambia has refused residence to any non-African who has not been immunized against yellow fever. The time seems now to have arrived when a similar ordinance might well be passed in other West African colonies as well as in Uganda, the Anglo-Egyptian Sudan and the Belgian Congo. The ordinance should include Syrians, Indians and others of Asiatic origin. In view of the particular dangers to which the medical and administrative staffs are exposed in dealing with outbreaks of yellow fever it would seem desirable that all medical, sanitary and political officers should be immunized in those countries where yellow fever is at present non-endemic but to which it might conceivably spread.

In view of the special position of the East African littoral in regard to the extension of the disease to Asia it would be desirable to undertake mass inoculation of the populations of the main ports, Mombasa, Tanga, Dar-es-Salaam and Lindi as well as of the population in the coastal belt of Kenya. At present, owing to the widespread endemicity of the disease mass immunization of Africans in West Africa would seem to be unnecessary unless fresh immunity surveys reveal that key points have a low immunity rate in comparison with surrounding areas from which they might be readily infected. Certain countries have seen fit to impose quarantine periods on persons who have been inoculated against yellow fever with the attenuated tissue culture virus. Laboratory experiments (ROUBAUD, STEFANOPOULO and FINDLAY (1937) and WHITMAN (1939)) have shown that *A. aegypti* does not take up the attenuated virus from the blood although the virus may be present in the blood stream in considerable concentrations. These experiments have been confirmed on a large scale in the field since some millions of people have now been inoculated in endemic zones in South America and Africa without giving rise to any epidemic of yellow fever. The imposition in fact of any quarantine at all on persons who have been inoculated longer than that imposed on non-inoculated persons who have come from an infected zone thus errs on the side of caution.

Since immunization was only instituted some ten years ago it is still uncertain how long immunity lasts. A number of persons however have continued to show immune bodies, 7, 8 and 9 years after immunization and

there is considerable evidence to suggest that once immune bodies have been demonstrated in the serum immunity may continue for a number of years, if not for life.

The intraperitoneal mouse protection test, it must be remembered, does not give an absolute zero since immune bodies may be present in the serum in amounts insufficient to neutralize the large dose of virus used in the test. If and when the mouse protection test fails to show the presence of immune bodies reinoculation is desirable. Tests for immunity should be carried out two years after inoculation; when such testing is impracticable reinoculation should be carried out every two years.

Immunity tests can be carried out in London, Lagos, Entebbe and Johannesburg as well, of course, as in New York and South America. In view of the increasing amount of work which these tests are likely to entail in the future it is desirable that laboratories in Khartoum, Nairobi and Dar-es-Salaam should also be equipped to carry out this test.

#### (4) *Administrative Action*

Various steps which require administrative action have already been mentioned. Attention may also be drawn to certain other points which require to be carried out by administrative authorities.

(i) Although any country in which a case of yellow fever exists must declare an infected area, the period after notification of the last case during which quarantine remains in force has been left vague. Thirty days would seem to be a suitable time to which all countries should adhere.

(ii) In view of advances in our knowledge of yellow fever the sanitary regulations in force in many of the West African colonies require fresh study and possible amendment. Thus since *A. aegypti* bites as readily in the late afternoon as at night the placing of infected areas in quarantine from 6 p.m. to 6 a.m. is hardly likely to aid in preventing the spread of infection.

(iii) At present a large number of countries both in Africa and Asia are interested in the question of yellow fever either because the disease is already endemic within their borders or may spread to them. Each country is solely responsible for public health measures within its border. Just as lack of co-ordination for defence against aggression has been one of the main factors in permitting the spread of the present war throughout Europe so the lack of co-ordination in measures against the spread of yellow fever may well be an important factor in aiding the spread of yellow fever throughout Africa.

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## DISCUSSION

**Dr. Hugh H. Smith:** It has been very interesting indeed to hear Dr. FINDLAY's account of the situation in Africa. I am sure we are all agreed that there is much to be done before anything like a true picture can be had of the epidemiology of yellow fever in that continent. I would like to say a few words about what has been going on in South America, particularly since Dr. SOPER gave his report before this Society in October, 1938. There has been, so far as I know, no outbreak of yellow fever in South America since then transmitted by *Aedes aegypti*, but we have had a great deal of jungle yellow fever.

For the last 2½ years I have been associated with the work in Colombia and since time is short it is better to confine my remarks to what has been done there. Colombia offers perhaps a unique opportunity for the study of yellow fever because there are a number of small isolated valleys both along the mid-portion of the Magdalena River and on the eastern side of the Andes, where yellow fever continues to recur. The epidemiology of the disease in

Colombia has been studied rather intensively since 1933 and cases have been discovered each year. It has been interesting to learn that yellow fever recurs in the same places frequently within a few hundred yards of where it was found previously. There are sometimes 2, 3, 4 or 5 years between outbreaks, but it continues to come back in the same narrow valleys.

There is a central laboratory in the City of Bogotá which is very high, almost 9,000 feet above sea level, and not at all suited for transmission experiments on insects. This laboratory serves as administrative headquarters for the service, for the examination of liver specimens, for testing sera and for the preparation of yellow fever vaccine. A field laboratory was built in 1938 near the small town of Villavicencio about 1,500 feet above sea level. For the last 2 years each time an outbreak of yellow fever has occurred we were able to put laboratory workers in the field with rhesus monkeys and white mice to endeavour to discover what was the vector in that particular outbreak. In spite of intensive effort in the midst of active cases we have not discovered any virus outside of humans except in mosquitoes. Between November, 1940, and February, 1941, virus was isolated eight times from the wild bush mosquitoes in the Villavicencio area. All these mosquitoes were species of *Haemagogus*. It has been very difficult in Colombia to explain what is the mechanism of a continuing yellow fever infection in these areas. In one or two instances we have found yellow fever in the bush far from human habitations. One instance occurred about a year ago when one of the oil companies sent a small company of geologists out to an area on the Llanos which was uninhabited by man. They had to cut their own trail through the woods. One of the men came down with yellow fever and was brought back by aeroplane to Villavicencio. A group from our laboratory went out by plane to study the situation. Although no virus was isolated from insects in the area, blood obtained from wild animals, especially monkeys, showed a high percentage to have antibodies against yellow fever. Dr. FINDLAY is therefore justified in saying that perhaps yellow fever can be maintained in the bush or jungle without human cases. There is no agreement among various workers in South America as to whether yellow fever infection remains in these particular endemic areas for long periods of time, and only infects human individuals at intervals, or whether there is some mechanism by which the infection travels continuously through the bush and returns to a given area periodically.

Facilities have been set up in Villavicencio for establishing colonies of various bush mosquitoes in the laboratory, in order that transmission experiments can be carried out under the best conditions, and also for studying the possibility of the passage of virus from the infected adult mosquitoes through the eggs to the next generation. It is known that in *A. aegypti* such a transmission of virus through the egg is not possible, but we do not know if it is possible with certain species of wild mosquitoes. We have made

a great many studies on various species of ticks and on other kinds of blood-sucking and non-blood-sucking insects to attempt to find a long-lived host for the virus; but all experiments have so far been unsuccessful.

It is worth while to emphasize what Dr. FINDLAY said about the great difficulty of making a clinical diagnosis of yellow fever. There are many places in South America where relapsing fever, malaria and yellow fever occur in the same area, sometimes simultaneously. It is almost impossible, no matter how expert may be the diagnostician, to differentiate with certainty between these diseases without the aid of laboratory tests. We have established the practice of only reporting to the Pan-American Sanitary Bureau in Washington cases confirmed by one of the three methods mentioned by Dr. FINDLAY. I believe that is the only reliable basis upon which to report yellow fever.

As to the prevention of the spread of yellow fever: it is known that by vaccination of the individual we can protect the individual, but I am not sure that even by the vaccination of a whole rural community you can prevent yellow fever spreading through that area—certainly not, if it can be spread, for example, by mosquitoes and animals without human cases. So it does not seem possible that we can prevent altogether the spread of jungle yellow fever in any area by immunizing people. Vaccination does, of course, reduce greatly the possibility of infected humans travelling from the epidemic area to uninfected regions. In Colombia we found that it is very difficult to vaccinate more than 65 per cent. of the rural population, even in the presence of yellow fever. In many communities where there were no actual cases it was difficult to vaccinate more than 20 to 25 per cent. of the rural population. It seems necessary, therefore, to continue the rigid control of *A. aegypti* breeding in all cities and towns anywhere near endemic areas of yellow fever. With the approved methods that have been worked out in Brazil, the cost of such mosquito control is not prohibitive. Dr. SOPER is now setting a goal for the complete eradication of *A. aegypti* in certain areas, and he is even speaking of eradicating the mosquito completely from the whole of the Amazon Valley. It remains to be seen whether or not that is possible.

**Col. F. P. Mackie** asked whether Dr. FINDLAY could clarify the conflicting statements regarding the development of immunity in inoculated persons.

The issue of certificates of inoculation to air travellers is at present complicated by the fact that no two countries are agreed as to the time which must elapse between the inoculation and the entry of the person into an infected area or into a non-infected country. The Anglo-Egyptian Sudan requires 10 days to elapse subsequent to inoculation before a passenger can enter the Sudan, whereas across the frontier the Egyptian authorities require a minimum of 21 days and a maximum of 3 years.

India by a recent notification has further complicated the position by requiring an interval of not less than 14 days before the passengers' entry into

## DISCUSSION.

a yellow fever area and not less than 23 days before his arrival in India. A maximum period of 2 years is required before the inoculation must be renewed. The confusion caused by these inconsistencies is putting strain on passengers by air and their advisers who are only too willing to comply with any reasonable demands.

It is now much simpler to obtain from a doctor a certificate that the passenger has not been exposed to yellow fever infection within the previous 6 days than it is to obtain a certificate of inoculation which seems to declare him a suspect.

**Col. G. A. Gill:** I would like to ask Dr. FINDLAY one question: has he correlated the time of the epidemic he described with the sun-spot cycle? In one of his previous papers he showed that all historic outbreaks of yellow fever in West Africa occurred somewhere about the time of sun-spot minimum, but this epidemic, I think, must have occurred at or near the sun-spot maximum.

**Dr. H. S. Stannus:** It would be interesting to hear what the vector was in the outbreak of relapsing fever, and whether affections of the lung and eye occurred as are seen in the tick borne disease. Also whether Dr. FINDLAY had ever had the opportunity of examining the serum of an African native stated to have had blackwater fever. Blackwater fever has been said to occur occasionally in natives but is it possible they have been cases of yellow fever?

**Brig. D. T. Richardson:** I want to know from Dr. FINDLAY if there is any relationship between yellow fever and dengue as regards the immune protection test? In Jamaica yellow fever was endemic for years and suddenly died out but the vector still remains and continues to be as abundant as ever. Dengue is epidemic in the island and troops on arrival are very susceptible, they do however become seasoned and the immunity probably lasts during the rest of their three years' tour.

**Lt.-Col. T. Menzies:** Could Dr. FINDLAY tell us anything about the latest method of immunization—scarification by a mixture of yellow fever virus and smallpox virus? It seems to me it would be useful to combine the two.

**Prof. T. F. Hewer:** There is only one question I would like to ask: whether Dr. FINDLAY came across any of the cases of acute necrosis of the liver that were common in various parts of the Sudan when I was there. Their clinical resemblance to yellow fever led to some confusion at that time.

**Dr. Hugh H. Smith:** I would like to say a word about the interval which is required after vaccination for immunity to develop. In Brazil, when studies

on the 17D strain of vaccine virus were begun in 1937. we bled a group of about twenty-five men at weekly intervals after vaccination and tested their sera in mice for antibodies. None of the group showed antibodies at 7 days; 60 per cent. of the sera gave definite protection to mice at 14 days; and all of the men had antibodies at 21 days. Rhesus monkeys inoculated with virulent Asibi virus at varying intervals after vaccination show evidence of increased resistance as early as the 5th day and are apparently fully immune by the 7th day after their immunizing dose of vaccine virus.

Practical experience with yellow fever vaccination of laboratory personnel and of rural populations in the presence of epidemics has convinced me that, in general, an effective immunity develops as early as the 7th day following vaccination. I think, therefore, that Dr. FINDLAY is quite right in saying that no more than 10 to 14 days should be required by health authorities before allowing the free movement of any vaccinated person.

**Dr. G. M. Findlay**, in reply, said it was now generally agreed that persons who had been inoculated against yellow fever were quite immune to the bite of an infected mosquito 10 days later. In addition after immunization with the attenuated yellow fever virus the virus circulated in the blood for only a few days and in any case extensive experiments had shown that *A. aegypti* could not become infected by feeding even when the blood did contain the attenuated virus used for immunization. In many hundreds of thousands of vaccinations there was no evidence that any epidemic had been initiated by the use of the attenuated vaccine virus. There was, therefore, absolutely no scientific justification for imposing quarantine periods of 21 days or over on persons who had been immunized.

It was impossible for anyone who had lived in an endemic zone to say that they had not been exposed to yellow fever and therefore certificates of "non-exposure" granted by any country where yellow fever is endemic were farcical.

So long as immune bodies could be shown to be present in the blood by the mouse protection test there was no need for reimmunization. If it was impossible to have a mouse protection test carried out it was advisable to be reinoculated every 2 years, although there was evidence to show that anyone who had ever had immune bodies in their blood retained some degree of immunity for many years. Combined inoculation by scarification of vaccinia and yellow fever was not recommended. Dengue and yellow fever gave no cross immunity. Cases of acute or subacute liver necrosis, associated with jaundice and black vomit, often caused confusion with yellow fever.

As regards the sun-spot question: I have not gone into the matter in connection with this epidemic.

## COMMUNICATIONS.

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### STUDIES ON *LOA LOA* AND THE FIRST REPORT OF *WUCHERERIA BANCROFTI* IN THE SUDAN.

BY

H. M. WOODMAN, M.B., B.CH.,\*

AND

AHMED BOKHARI, D.K.S.M.

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Though the vague condition "filariasis" has been known for many years in the Sudan, it is only in the last decade that work has been done to discriminate the filariae involved and the influence these have on the health of the indigenous population.

It was in the course of concentrated study of *Loa loa* that *Wuchereria bancrofti* was discovered for the first time in 1937.

\* We are indebted to Dr. E. S. HORGAN, Assistant Director of Research, Khartoum, for assistance in arranging this paper which is in the nature of a preliminary note as the exigencies of war have separated the authors from their records.



The occurrence of the nematodes *Acanthocheilonema perstans*, *Onchocerca volvulus* and *Loa loa* are well known, and their range is familiar throughout the equatorial (old Mongalla and Bahr el Ghazal) province of the Sudan. *A. perstans*, in spite of its incidence in over 50 per cent. of the population in certain parts of the province, and MOLSER'S (1939) rather doubtful statement that it causes fever of 38° C. and 39° C., remains as far as all observations have shown, an innocuous and harmless parasite. The very important lesions, including "Sudan blindness," certain eye, skin and nodular phenomena attributable to *O. volvulus* have been described by HISSETTE, CRUICKSHANK and BRYANT (1935). Attention was drawn to the remarkable prevalence of *Loa loa* in the south-western districts of the province and the adjoining territories of the Belgian Congo and French Equatorial Africa by WOODMAN (1935). First acquaintance with *Loa loa* disclosed an interesting problem, the main factors of which can be summarized as follows:—

Firstly, certain morphological features of the microfilaria, its periodicity and clinical phenomena, did not seem to conform to the classical account of the West African variety.

Secondly, only one of the species of *Chrysops*, described as vectors of *Loa loa* by the CONNALS (1922), had been found in the neighbourhood, and this in one confined corner of the district only.

Corresponding with this incidence of *Loa loa* were the familiar lesions of bancroftian filariasis. They were by no means prevalent but, nevertheless, cases of elephantiasis of the scrotum, penis, labiae, or leg were fairly common. Although it was established that *O. volvulus* was capable of giving rise to these lesions in parts of the area studied, this filaria was so rare in others that another causative factor had to be sought.

The orthodox and commonest cause of elephantiasis—namely, *W. bancrofti*—had been searched for in the Sudan without success. The only record of its occurrence was in a slide made by Sir ROBERT ARCHIBALD on the Abyssinian border before the last war. This was not published and the particulars were lost. As it was so rare, and had never been seen west of the Nile in the Anglo-Egyptian Sudan, the interesting third point therefore arose: were these phenomena attributable to the Central African variety of *Loa loa* or possibly to a new filaria with which this was being confused? An adult worm closely resembling *Loa loa* had incidentally been reported from a human being by MAPLESTONE (1938) in India.

#### LINE OF INVESTIGATION.

*Loa loa* was closely studied in relation to its vector in which the development of the embryo was followed through all its stages. Certain other tabanids were also fed and dissected, while 400 wild chrysops were examined for naturally acquired infections.

The morphology and periodicity of the microfilaria were investigated, and the nematode was found in certain lymphangitic lesions.

Cases of known *Loa loa* infection with Calabar swellings, eosinophilia, presence of the worm in the eye, etc., were studied in detail, while various drugs were tried *in vitro* and *in vivo* with a view to influencing the microfilaria.

The presence or absence of *W. bancrofti* was determined by careful staining and examination of day and night slides of bloods infected with sheathed microfilariae.

Ground doves and other birds, were examined for the presence of filariae.

#### GEOGRAPHICAL SITUATION.

The district in which these observations were made is in the Southern Anglo-Egyptian Sudan, between the frontiers of the Bahr el Ghazal and the Belgian Congo, between latitudes 4° and 6° N., and longitude 27° and 31° E. The main features are open savannah forest, a rainfall of approximately 51 inches, an altitude of 2,000 feet, and a temperature range from 54° F. minimum to 100° F. maximum. The area covers 15,000 square miles and carries a population of over 120,000.

The people are entirely of the mesaticephalic Azande tribe, or its partially absorbed subordinates numbering nearly ten ethnologically related peoples. They are purely agricultural, living on a carbohydrate diet based upon eleusine, manioc, maize, ground-nuts, bananas, beans, and forest roots and herbs. They have no meat except in the short hunting season, and are completely deprived of milk. There is no written record of these people earlier than the eighteen forties. The district was served by three hospitals.

#### *Wuchereria bancrofti.*

After more than 1,500 films of day and night blood of cases showing sheathed microfilariae had been examined, the first clear instance of *W. bancrofti* infection was demonstrated. Some dozen cases were subsequently found between the years 1937 and 1938. The microfilariae had the typical morphological appearances and the identification was confirmed without hesitation by Professor R. T. LEIPER when they were shown to him. (Microphotographs are displayed in the Khartoum Graphic Museum.) Three cases of the day or non-periodic type were observed; the remainder showed nocturnal periodicity.

More often than not there was a mixed infection with *Loa loa*. No adult filariae have been found. No actual cases of elephantiasis, lymphatic varix, varicose groin glands, lymph scrotum or hydrocele revealed either the microfilaria or the adult. Elephantoid fever was never seen nor was acute lymphangitis of filarial type encountered.

Hydrocele fluid was centrifuged and direct smears made from the walls of the tunica vaginalis at operations. Though in one case *O. volvulus* was found, *W. bancrofti* was never seen.

The fact that the adult has so far escaped discovery is the more remarkable in the light of O'CONNOR's observation that 75 per cent. of the adults are to be found in the lymph vessels round the glands or in the capsule and cortical sinuses of the glands, and that the most common situation for the worms is the scrotum.

#### INCIDENCE OF *Loa loa*.

Fifteen per cent. of the population showed *Loa loa* in the blood. Added to these must be included those "hidden" cases which have had, or will have, microfilariæ at the moment optimal to the parent worms which they carry—these latter from time to time coming to light at surgical operations in spite of negative blood films. Probably a 20 per cent. infection of the population would be a conservative estimate.

Twenty-three per cent. of British and Syrian officials of this part of the Sudan were known to be infected with *Loa loa*, and the incidence in the North-Eastern Congo is probably still heavier among European residents. The rivers Bomakandi and Nepoko in the mines area of the latter are particularly heavily infected.

The vector, *Chrysops silacea*, Aust., was only known in one corner of the district (Source Youbo) and only in appreciable numbers in one year out of five during the rainy season, when it was taken on cattle.

*Chrysops dimidiata*, Van der Wulp, was not found, and is believed not to occur.

*Chrysops distinctipennis*, Aust., was much the commonest; it was evenly distributed throughout the western two-thirds of the district, and occurred at all seasons.

*Chrysops longicornis*, Macq., was comparatively uncommon and found in the proportion of 1 : 26 of *C. distinctipennis*.

The habits of the last two were furtive and, rather than being voracious where man was concerned, they were seldom noticed to attack human beings. Most natives when questioned said that they did not consciously remember being bitten. They knew the flies well, having different names for two of the species. The flies were hard to find in appreciable numbers except near cattle. In wet weather they were sluggish, almost clumsy in flight, improving when the sun was well up and in the hotter hours of the day, but their vehemence was never comparable with *Haematopota*, *Stomoxys* and *Glossina*. The year that *C. silacea* was found it was noted that it was rather more aggressive to man than the other species.

It was decided to repeat the CONNALS' experiments, with minor modifications in feeding and dissecting, with *C. distinctipennis* and *C. longicornis*.\*

\* I am indebted to Mr. W. RUTTLEDGE, Government Entomologist, for determinations and much practical advice and assistance in handling the fly and finding means of making the experiments a success.

In the short season when *C. silacea* were collected there was no opportunity to carry out dissections. Accordingly, the flies were preserved in Carnoy's fluid in the hope of carrying out section examinations at a future date.

Six hundred wild *C. distinctipennis* were dissected and four were found to be carrying embryos of *Loa loa*.

Three hundred experimentally fed flies were dissected, the following being an outline of the technique.

Wooden frame boxes, 6 × 6 × 6 inches were made, covered in with panels of thin native cotton cloth, leaving a sleeve on one side for inserting the hand. A tin floor was included on which damp sand was placed. Blades of coarse grass were stood on this upon which the flies could rest. One side was covered with copper mosquito wire and turned to the window. It was thus simple to remove flies from the sleeve behind with a test tube while they crawled on the wire on the opposite side. It was found that these cages did not require moistening by gauze wick from a water reservoir, as was tried at the commencement of experiments, for they were sufficiently damp without the aid of this device.

They were kept in a laboratory where temperatures seldom rose above 90° F.; humidity was high, and air freely circulated.

In this way flies were kept alive in extreme instances up to 24 days; many lived to 18 days, the remainder died at varying dates from the 4th to the 14th day.

Much difficulty and patience was required to induce the flies to feed. They were given an opportunity to feed by being lightly held in an inverted test tube over the bare skin. They were moved with great care to avoid any bruising. They were offered meals on normal native skin, on skin cleaned with ether (all trace being removed by washing afterwards), on skin treated with extract from the hide and hair of cattle upon which the fly was found. Different hours of the day were tried, in heat and in shade, but under no conditions, with very rare exceptions, was there any keenness displayed. Exceptionally, the fly would feed at once. Sometimes after waiting up to 20 minutes an inclination to feed would appear. More often a fly would have to be tried morning and evening before feeding and frequently offered a meal on two or more successive days before success could be achieved. A number of flies, kept up to 5 days, would persistently refuse a meal and they consequently died. Flies which had obviously already fed when caught were discarded.

Those flies which lived more than 10 days had been successfully induced to take a second feed which was always offered them from the 7th day onwards. Those which survived more than 14 days had successfully taken a third feed, offered from the 11th or 12th day onwards.

Flies were killed by chloroforming, the dissections being made at all stages from the first few hours up to the 24th day.

Development of the embryos took place along the lines described by the CONNALS (1922), but more slowly. By the 5th day embryos were in the CONNALS' 2nd day stage, by the 8th day in the 3rd day stage, and so on. Another notable difference was the irregularity. Some reached apparent maturity on the 12th day, others on different days up to the 16th day of the fly's survival.

After this date there was no further change in the embryo. In others the development was still slower, and in a considerable percentage it was irregular, the majority of embryos developing at the same stage but a smaller proportion lagging some days behind.

No fly, carrying mature embryos after the 12th day, would accept a re-feed, which was disappointing as it was thus impossible to observe the filaria leaving the labium to attack the skin.

The concentration of embryos was almost exclusively in the abdomen up to at least the 9th day and the ovaries were the favourite site. Later they might be found around the midgut and accessory body, and a few would migrate into the thorax. On reaching maturity they concentrated in the head.

The features of special note compared with the CONNALS' experiments are :—

(a) The inaptitude of *C. distinctipennis* and *C. longicornis* to feed on human blood. (b) Their relatively furtive habits. (c) The longer and often irregular development of the embryo and its comparative immotility. (d) The longevity of the fly when kept in captivity.

The flies were fed on volunteers whose blood was infected with *Mf. loa*. The very delicate and difficult operation of breeding the flies in the laboratory for these experiments was not attempted. The fact that less than 1 per cent. were found infected in the wild state was deemed not high enough to affect appreciably the observations made.

Flies which were re-fed were also offered their meal upon infected volunteers. The development being so slow that there was no fear of confusing the later brood of embryos with the former when the fly was dissected. As would be expected no fly lived long enough for a second meal of embryos to reach maturity.

#### EXPERIMENTAL VECTORS.

From the characters of the *Chrysops* spp. just described the inference was that, while these flies could and did carry *Loa loa* infection, it seemed probable that other vectors must exist.

Selections of the blood sucking tabanids, commonest in the district, were caught, artificially fed, and dissected on the same lines as in the chrysops experiments. These were the following :—

*Haematopota decora*, Walk.

*Haematopota vittata*, Lw.

*Haematopota brummiscens*, Ric. (at Meridi).

*Stomoxys calcitrans*, L.

*Stomoxys nigra*, Macq.

*Glossina morsitans*.

*Glossina palpalis*.

It had been noted by LEIPER in Nigeria in 1912 that partial development might occur in *Haematopota*. Accordingly, forty specimens were fed and caged and were not dissected until they had reached a dying condition. Most died before the 10th day, but one lived to 16, one to 18 and three to 12 days. None of them showed embryos of more than 7 days' development (corresponding to the 3rd day stage in the CONNALS' account) and when surviving more than 10 days the embryos were non-motile, if not actually dead.

The results with the four last, twenty of each of which were dissected, were entirely negative.

The only common blood suckers of man other than the above-mentioned biting flies were mosquitoes, *Simulium* and *Culicoides*. If a parallel was to be made with the life histories of other nematodes and their hosts, it seemed improbable that members of two different families would be natural carriers of the same parasite. Moreover, the size of the mature embryo (more than twice that of *W. bancrofti* and *A. perstans*) would render its delivery from the labium of these smaller insects almost a physical impossibility. Four *Taeniorhynchus africanus* were actually fed, and dissected on the 3rd day, with negative results. But in view of the discovery of *W. bancrofti* in the district and the relation of it to the many *Culex*, *Aedes* and *Anopheles* capable of carrying it, it is not impossible that partial or even complete development of *L. loa* may take place in one or more of them. That the principle was not impossible had been already shown by BREINL (1921), who demonstrated the complete development of *Dirofilaria immitis* in the dog flea. Fleas seemed to bear no similar relation to *W. bancrofti* development, although the low infection rate among the recognised mosquito vectors of a certain area where bancroftian filariasis was prevalent was drawn attention to by YOKOGAWA (1939) and seemed to suggest the occurrence of other hosts.

YAO, WU and SUN (1938) have also shown that *W. bancrofti* can develop in species of sandflies, *P. chinensis* and *P. sergenti mongolensis* being found infected in the wild state, while experimentally fed flies showed a degree of development which might have been completed had they lived.

#### IDENTIFICATION OF *Loa loa*.

There was no doubt of the recognition of adults of *L. loa* found in the tissues at operations. Certain divergences from the typical appearances in the microfilariae at first lent colour to the view that an undescribed variety of sheathed embryo occurred. The main points were:—

The nuclei of the central column of cells were notably larger and more prominent in the first two rows of the cephalic end. The first row was often of three long nuclei roughly parallel but showing variation in their position in relation to one another, a lateral one usually being furthest forward. It was not uncommon to find one or more of these well forward, leaving very little

of the typical clear space, that faintly stains without nuclei, of the classical descriptions.

The dispositions, though often irregular and thus typical, were also quite often in neat circles.

The tail tip was long, tapering and fairly frequently, but by no means always, typically flexed. The nuclei varied considerably in how far they extended into the tail in a single line. (There was never any disposal of two detached nuclei in a background faintly stained towards the tip, as in *Mf. malayi*).

The sheath occasionally took up Geimsa's stain.

Relative measurements from the anterior V spot to the cephalic tip, and from the V spot to the tail spot, showed divergences from the classical textbook measurements.

#### PERIODICITY.

Comparison of 72 night slides with day bloods taken in the same cases showed that the microfilariae were not diurnal. In some they were present in equal numbers at all hours but usually fewer were seen at, say, 9 p.m. Sometimes none were found at all at 10 p.m., while they appeared again by midnight. In only 9 per cent. of cases were the microfilariae absent entirely up to 12 p.m. Bloods that swarmed by day usually showed a clear reduction in numbers at night.

Diurnal periodicity has been shown by SHARP and others not to be a characteristic of *Loa loa*, and these observations could only be considered to bear that out rather than suggest that we were dealing with a different microfilaria.

With the kind assistance of Professor LEIPER, slides were compared in London with specimens from other parts of Africa, notably the Gold Coast and French Equatorial, and it was shown that the measurements were not incompatible with other strains of *L. loa*. The atypical features had been tabulated whenever a slide had shown them, and they were the exceptions. Moreover, they had been picked out of some 1,500 blood films. While emphasizing that *Mf. loa* could show a possibly wider range of size, disposition and shape than had been described before, taking the facts by and large, it seemed clear that we were only dealing with *Loa loa* and no new microfilaria.

It was interesting to record in passing that the average number of microfilariae per field in a typical thick blood film, under a  $2/3$  lens and a 4 eyepiece, would be considerably less than one by day. But one case was as high as thirty-one and another fifty-four. This latter was in a man aged about 38, who demonstrated this swarm of microfilariae over the whole 2 months he was under observation. There was no history of swellings, glands, worm in the eye, or other clinical manifestation of loiasis, but he was blind. At the time of examination he had early cataract but the history was of rapid onset. Whether there were posterior ocular changes like those caused by *O. volvulus*,

and if the condition was induced by the mechanical or pathological effects of *L. loa* was impossible to say. The youngest subject found infected was only 4 years—the oldest 56.

#### REACTION TO DRUGS.

Writing from memory, no precise details can be given of drugs used and observations made except that they included acriflavine, various aniline dyes such as methylene blue, trypan blue and fluorescein; also antimony and mercurochrome. Results were all negative except in the case of methylene blue, where mf. counts were done periodically. This drug was given in courses intravenously beginning with 2 c.c. of 5 per cent. solution and working up to 5 c.c. of 5 per cent., previous experience having been gained in its use in large scale treatment of leprosy (WOODMAN, 1937), the racial reaction seldom giving rise to the toxic symptoms of headache, backache, giddiness or syncope sometimes said to be associated with it. The influence of this drug in a 1:5000 solution *in vitro* was pronounced, as described by SHARP (1923). There was also a reduction in microfilaria counts after injections in these cases, but controls showed so much variation in numbers circulating in the blood at different times that no conclusion could be deduced that the drug had any beneficial influence *in vivo*. MOLSER (1939) had claimed that *A. perstans* disappears from the blood with intravenous injections of methylene blue, using 2 c.c. up to 10 c.c. of 1 per cent. solution. This was contrary to our experience with either *A. perstans* or *L. loa*.

ADAMS (1938) found that the microfilaria of *O. volvulus* can be reduced in number by plasmoquine and neostibosan with a temporary reduction of eosinophilia, but the adult was not affected. A patient developing Calabar swellings, after returning to Europe, was given eleven injections of anthiomaline by DE CHOISY (1937), up to 3 c.c. of 6 per cent. solution on alternate days. The recurrence of swellings was reduced to two per month and then none at all. There was no doubt that if given in large enough doses methylene blue would be able to destroy the parasite, but as HISSETTE (1938) concluded after reviewing the whole problem of onchocerciasis in Africa and Central America, it was doubtful if any drug would be found to destroy the microfilaria of this or any other species which would spare the host.

The whole study of the reaction of the reticulo-endothelial system to *Wuchereria bancrofti*, and in varying degrees with other filariae, by CLAYTON LANE (1937), and how the mobile cells of this system become blocked by repeated injections of dyes, gives confirmatory evidence in support of this view. The defensive mechanism of these cells is thrown out of action by their becoming overloaded with dye which renders them immobile and inactive. (HADFIELD and GARROD, 1934.) The work of KHAW and CHEU (1936) on *D. immitis* and NOC (1923) on *W. bancrofti*, working with fouadin and amino-arsenophenol, respectively, show more hopeful prospects for sterilization of the adult females,



if not destruction of the worms. One cannot regard the trial of methylene blue as complete until its influence upon the parturition of the adult in the tissues has been seen.

### SYMPTOMS.

The usual phenomena of Calabar swellings, high eosinophilia, and microfilariæ in the blood were noted, as in the classical accounts. There also seemed some ground for supposing that *L. loa* was a factor in certain organic pathological changes, notably hydrocele, hyperplastic lymph nodes, lymphocele, and a disposition to inguinal hernia.

### *Calabar Swellings.*

Six typical cases were observed in native hospital staffs (totalling some seventy-five individuals) in the course of a year. It was impossible to elicit clear histories from natives in whom transient swellings might have occurred, therefore only those actually seen were diagnosed. In the case of one European the swellings first started simultaneously in both feet. A month or so later—in one forearm, where the tensity and size of the swelling had the appearance of acute sprain with teno-synovitis. There was no pitting on pressure, it was hot to the touch, lasted 5 or 6 days, and gradually subsided, leaving a transient brownish discoloration in the skin. There was considerable discomfort from the tensity of the swelling. A worm had been noticed passing over the knuckle of the hand within 12 hours preceding the reaction.

During the subsequent 12 months the worm was felt or seen in the conjunctiva, in the eye-lid, face, loins, leg or foot on numerous occasions, and accompanied by intense itching sensations. The first swelling appeared after the subject had had rather less than 4 months' exposure to the endemic area. The first adult worm was seen 7 months later. No microfilariæ have yet been seen in the blood, after 7 years. It was noted that if a worm was seen near the surface that a swelling invariably followed within 12 hours. This could be reduced with ice packs. It was also noted that the worm would recede after contact with the ice pack, and that if applied at once the swelling could be at least partly aborted. After the first contact with a European climate and during subsequent visits to the tropics, the swellings became more infrequent, considerably smaller and, after 2 years, were not preceded by conscious movements or appearances of the worm. After the first 3 years there was no recurrence of swellings for 4 years, when one appeared again. The interesting points about the case were (1) The appearance of Calabar swellings after less than 4 months' exposure. (2) The absence of microfilariæ even after 7 years. (3) An eosinophilia of 73 per cent., the highest seen in a long series of cases, and comparable with films demonstrated by ADAMS before the Royal Society of Tropical Medicine in March, 1938, from a European who had had 10 years in

West Africa. This case showed 84 per cent. eosinophilia. He had had Calabar swellings for the last 5 years.

Another phenomenon of note in Europeans was a sensation of movement in the lumbar region, followed by a "lumbago" characterized by inability to stand up straight for 24 to 48 hours. It usually recurred in the same place and in the same way and came on perhaps half a dozen times a year. This was in a man of 45 who had had a long-standing *L. loa* infection for an unknown number of years and who no longer had Calabar swellings.

Another individual—a man of 40 who had had 5 years in the district—showed a leathery, slightly indurated, condition of the skin of one forearm with faint discoloration and general enlargement. Sensation was unimpaired. It was associated with an eosinophilia of 20 per cent. There was nothing in the faeces or urine, and nil in the history other than chronic malaria, and no microfilariae in the blood. The condition had cleared up after six months, after which I lost touch with him. It was thought to be a possible *Loa loa* infection. Only one of the cases of Calabar swellings in natives showed microfilariae in the blood. They were all conscious of moving adults from time to time.

The highest eosinophilia noted was 73 per cent. as recorded above. The average was 13 per cent. and the highest in a native 43 per cent. All showed some degree of leucocytosis, averaging 14,000; the highest being 33,750 (with an eosinophilia of 30 per cent.). Calabar swellings seemed to occur in relatively early cases as far as could be judged, and these were the ones showing highest eosinophilia counts. A very comparable eosinophilia in onchocerciasis was found by HAWKING (1939) in Kenya, with an average of 15.2 per cent. and a maximum of 49 per cent. In the Sudan, neither schistosomiasis nor ancylostomiasis, uncomplicated by filariasis, showed such degrees of alteration in the differential count.

The adult worm was seen crossing the conjunctiva in eleven cases of native and one of Europeans. It was noticed that they were easily killed by novocaine, when used as a local anaesthetic; one in an eyelid was killed and became cretified after a novocaine injection.

#### *Hydrocele, Lymphocele, Hernia and Lymph Glands.*

Hydrocele was the predominant surgical condition of the race; 80 per cent. of all operations performed at one of the hospitals (Meridi) were for hydrocele; 240 were radically treated in 1 year. What was the cause of this?

It could be argued that if *W. bancrofti* and *O. volvulus* had been proved to exist in the district these should account for the hydroceles and elephantiasis cases. The fluid from these hydroceles was nearly always clear and straw coloured with an occasional epithelial cell or leucocyte. A few were haematocoeles and contained chocolate brown or dark red coagulated material. The only ones showing chylous cloudiness were found as the common complication of elephan-

tiasis of the scrotum. Nearly a hundred fluids were centrifuged and examined for microfilariae together with a smear from the walls of the tunica vaginalis and a teased-out piece of adjacent connective tissue in each case.

To find microfilariae at all was rare, confirming KNOTT's work (1939) and ROMITI's (1935). In only one smear was the microfilaria of *O. volvulus* found. There was no suspicion of *W. bancrofti* even in the chylous fluid of elephantiasis hydroceles. Occasional microfilariae of *Loa loa* were found in the tissue or smears, more rarely those of *A. perstans*. The types of hydrocele were exactly like those described by KNOTT in the Virgin Islands, 1939, and are best summed up in his words "Filarial hydroceles differ greatly in morphology. Some are thin walled, flaccid sacs. Others are thick-walled and tense. They have many shapes. Some enlarge rapidly and steadily, some remain stationary in size for long periods and then suddenly begin to enlarge again. After tapping some refill quickly, others slowly. The tense, thick-walled hydrocele frequently becomes a haematocele . . . the lining membrane of thin walled hydroceles may show irregular whitish sclerotic plaques. One will find rust-coloured areas and small elevated brownish wart-like elevations. Calcification of old hydrocele sac walls is not uncommon."

Native policemen, transferred from relatively non-filarial areas east of the district under discussion, would sometimes acquire hydrocele and attend for examination after being a year or more in this district.

Inguinal hernia was the second commonest surgical condition, occurring as it did in a robust and virile tribe notorious for its thickset physique and former warlike qualities. At operation, it was common to find adult *Loa loa* moving in the tissues of the inguinal region, most particularly near the lymphatics and vessels of the cord. Smears from the exposed tissue would very commonly display the microfilariae. All cases demonstrating them in the blood would also show them in these tissues. Other cases revealed them in the tissues only. Enlarged soft lymph glands were common in the canal and numbers of them were excised and sectioned for examination.

At these operations varicolympheces of the spermatic cord were very commonly found, and these ranged from fine tortuous threads to thin-walled vessels a few millimetres in diameter. Some were supported in a bed of gelatinous, oedematous matrix characteristic of elephantiasis and in this stage the process seemed to remain stationary. It did not go on to enlargement of the epididymis and was not usually elicited by palpation before operation.

It was interesting that an adult *Loa loa* was found on three separate occasions in this lymphatic varix and, when present, was commonest in this vicinity. On no occasion was the adult or offspring of *W. bancrofti* found. Again, speaking without our records, I believe a microfilaria of *O. volvulus* was found once at this site. ROMITI (1935) described the clinical varicolympheces as a constant first manifestation of filariasis. The condition found here seemed to be the earlier stage of the same process, the difference being mainly one of degree.

The absence of *W. bancrofti* and *O. volvulus* and the demonstration of *Loa loa* was significant, and suggested implication of the latter as the main cause of the change.

It seems to us a possibility that *Loa loa*, and probably filariasis of all types, may be a factor in predisposing to hernia. The distribution of *L. loa* in central Africa seems to correspond to areas where inguinal hernia is most prevalent. This applies to the Southern Sudan, and Professor DUBOIS (at Pawa) and Dr. MONTCAREY (at Kilo-Moto) confirmed this in the N.E. Belgian Congo, where *Loa loa* and, to a less extent, *O. volvulus*, both occur throughout the northern mines area and the Ituri, south and east of the latter—both are rare and hernia less frequent. There may be something in KNOTT's (1939) theory of a constant enlargement of the lowermost iliac lymph glands, in company with the general reaction of the inguinal groups, and that these being imbedded in a soft varicose mass become more mobile and can easily get pushed through the internal abdominal ring and thus precede and predispose to a hernia. The relative lymph stasis and partial blockage of flow mechanically induced by the predilection of *Loa loa* for this area could in itself relax the inguinal rings in the course of time.

### *Elephantiasis.*

Elephantiasis occurred in the district but was not very common. An average of six cases would be admitted per annum in the three hospitals, averaging a total of 250 occupied beds between them. They were all in the scrotum, vulva, or leg. As mentioned before, there were no recorded instances of elephantoid fever or attacks of filarial lymphangitis or abscess. No cases showed adults or microfilariae of *W. bancrofti*, although it would have been reasonable to expect to find them in 20 per cent. of cases as recorded by MANSON-BAHR in Fiji.

The enlarged lymph glands taken from simple hernia operations of *L. loa* infected patients showed :—

(a) A slight degree of occlusion of the lymph vessels by endothelial proliferation.

(b) The presence of microfilariae in only two out of twenty sections, and round these there was no fibroblastic reaction or presence of giant cells in any way comparable with CLAYTON LANE's (1937) description of the macrophage and reticulo-endothelial reaction caused by *W. bancrofti*.

(c) The enlargement of the glands was caused by simple chronic fibrosis.

There was no "phagocytic filtration," as evidenced by DRINKER (1935) before with *D. immitis*.

Unfortunately, no lymph nodes from cases of elephantiasis were sectioned to make comparison with the above.

The occasional cretified worms found at these operations gave rise neither to lymphangitis nor lymph varix.

The evidence of these largely negative findings seemed to indicate the presence of a very mild pathological reaction comparable with the earliest stages of bancroftian infection and which seldom, if ever, continued to develop. There was no evidence that either the microfilariae in lymph glands or the cretified adult would give rise to (a) lymphangitis and consequent precipitating elephantiasis by rendering the tissues "some millions of times more susceptible to infection with haemolytic streptococci." (DRINKER, FIELD, WARD and LYONS, 1935.) (b) A lymphangitis following mass migrations of enormous numbers of sheathed microfilariae through the walls of the lymphatics to the blood stream (and such immigrations must periodically take place in instances such as the patient cited above with 54 microfilariae per field in an ordinary thick blood slide) as is said to occur with *F. malayi* by POYNTON & HODGKIN (1938); or (c) reaction of the reticulo-endothelial system in lymph glands at all comparable with those described by CLAYTON LANE in filtrations by *W. bancrofti*. In which latter case major fibroblastic change would take place eventually causing a damming of the lymph flow.

But the occurrence of early lymph varix was common and a general enlargement of the inguinal lymph nodes usual in cases of *L. loa* infections.

Our belief was that *Loa loa* caused these changes. It was presumed that the grosser lesions of elephantiasis and lymph scrotum were either caused by it as an exception and when a prolonged, localised, lymph stasis and lymphocele of the cord would render the tissues less resistant to streptococcal attack or that they were all accounted for by *W. bancrofti* and *O. volvulus*. The fact that neither of the latter, though diligently searched for, were ever found in elephantiasis cases would not be sufficient to preclude them. Further west at Wau, where *O. volvulus* was common, the few cases of elephantiasis there had been ascribed to this filaria, which was also generally thought to be the cause in the Belgian Congo.

#### FAUNAL AND AVIFAUNAL FILARIA.

The discovery of filaria in the ground dove *Columbigallina passerina nigrirostris*—by O'CONNOR & BEATTY (1938) in the Virgin Islands, led us to look round for bird and animal infections in the Southern Sudan. As one of us (H. M. W.) was collecting certain birds for the British Museum, it afforded an excellent opportunity (in 1938) to take slides.

The blood of the common dove, *Stigmatopelia senegalensis equatorialis*, was found to be heavily infected with microfilaria, and dissections revealed one adult in a chamber of the heart in one instance. No adults were found in the lungs or abdominal viscera and the livers were not enlarged. Twenty of these doves were examined and about 25 per cent. found infected. Two varieties of unsheathed microfilariae were seen.

The rare parrot—*Poicephalus crassus*—was also found to harbour another kind of unsheathed microfilaria.

## SUMMARY.

The commonest *Chrysops* vector of *Loa loa* in the Southern Sudan has been demonstrated and the course of the embryos' development is described in outline after 300 dissections. Comparison with the CONNALS' experiments with infected *Chrysops silacea* and *C. dimidiata* in West Africa showed certain modifications both in the development of the embryo and in the habits of the fly.

Some facts are put forward from which there is ground for the inference that vectors other than *Chrysops* also exist in Central Africa.

The occurrence, though rare, of *W. bancrofti* in the Sudan is reported for the first time.

The failure to find *W. bancrofti* at all and *O. volvulus* very rarely, in association with elephantiasis and hydrocele in this part of the Sudan affords some justification for attributing at least the earliest stages of lymph varix to *Loa loa*. Attention is drawn to a possible association between this filaria and the disposition to hernia and hydrocele.

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THE SEASONAL RHYTHM OF A FLY (*SPANIOTOMA MINIMA*)  
AND SOME THEORETICAL CONSIDERATIONS.

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*Spaniotoma minima* Mg. (Chironomidae) is a small black midge which breeds abundantly in some sewage bacteria beds, where it plays its important part in keeping the beds from choking and at the same time reduces the numbers of the annoying filter fly, *Psychoda alternata* Say, both by competition for food and by direct attack (LLOYD *et al.*, 1940). The insect has been watched in a general study of the fauna of these beds which has been in progress at Leeds University for some years. Insects emerging from the beds have been trapped by means of a wooden tray 1 square foot in area inserted into the bed surface and leading by a small opening and a paper cone into a glass jar. The jars are changed twice weekly and the trays moved to fresh sites once a week. The principle of the trap depends on the natural movement of most insects on emergence into the light to effect mating and dispersal. As *S. minima* mates only in an open dance the movement in its case is absolute and all those emerging under the tray, apart from such as may creep under its edges, enter the jar and have no impulse to return to the beds. Thus the



trap gives a very fair assessment of the rate of emergence from the area covered. Three traps are in use, sited some 35 yards apart on a long bed, and the figures dealt with are the weekly totals of the catches, the sum of six assessments. A daily record of temperatures in the depths of the beds and below a slate at the surface are kept. The bed temperature through the depth is fairly uniform and is relative to the air temperature as an air current penetrates the bed. Frost rarely penetrates and in summer it is cooled somewhat by evaporation. These conditions allow *S. minima* to emerge the year round and it has been absent from a trapping only very rarely. Its responses to temperature have been determined (LLOYD, 1937).

When the monthly totals of the fly are plotted as percentages of the year's total catch a characteristic curve of incidence results (LLOYD *et al.*, 1940). The fly is at a minimum in April, increases to a maximum in August or September, when 1 square foot of bed may yield several thousands a week, and then declines rather slowly. When its numbers are plotted on a weekly basis the curve is marked by peak periods of output, low and obtuse in winter, abrupt and very steep in summer, abrupt and with a tendency to be flat topped in autumn, the whole rather resembling the chart of a prolonged case of relapsing fever. The present study concerns the meaning of these peaks in which there is evidently a periodicity which is less than that required by a complete life cycle. The fluctuations are influenced by temperature in three distinct ways.

Firstly there is an effect of surface temperature which can make itself felt only when this falls below 50° F., the approximate minimum for the mating dance. Study of the charts with the average weekly maximum surface temperature superimposed suggests that a repressing effect of surface cold does not make itself felt above 47° F. at which heavy catches have been made while total repression does not occur till the temperature falls nearly to freezing. In this record the fly catch can be tested against surface temperature in fifty-nine weeks when it averaged less than 50° F. In thirty-three cases the numbers trapped followed temperature and in twenty-four the reverse occurred while three times the catch rose or fell with a steady temperature. Thus even in cool and cold conditions there is some other influence present apart from a temperature-activity response.

Secondly, there is the effect of bed temperature as it hastens or slows down the emergence rate from the pupae. This is more important and can show itself in any weather except the very coldest. Study of the charts of incidence against the weekly average bed temperature, the best criterion, shows that often the fluctuations of fly follow this closely, but many exceptions occur; 168 tests are possible and fly catch follows temperature in ninety-seven, in 58 weeks the reverse occurs, while in 13 weeks the temperature is steady and the catch rises or falls. Since the bed mean is related to the average weekly air temperature similar responses generally coincide but the former is the more important.

The reason for the numerous exceptions, discounting the element of chance, is obscure and once exerted can make itself felt through many succeeding generations. It has become evident through studying the curves of incidence by means of the progress-temperature law. For details of this the writings of UVAROV (1931) or of IMMS (1931) should be consulted. Briefly this law postulates that for each stage of an insect there is a temperature threshold and a thermal constant that can be expressed in day-degrees, the summation of the daily means above the threshold. These constants can be found by breeding the insect at two favourable distinct temperatures and timing the stages. Then if the temperature of the environment is known the time for the complete cycle can be deduced. For *S. minima* the theoretical thresholds and thermal constants are as follows (LLOYD, 1937): maturation of the female,  $32^{\circ}$  to  $113.5^{\circ}$ ; incubation of the egg,  $39.5^{\circ}$  to  $68.5^{\circ}$ ; growth of the larva,  $38.5^{\circ}$  to  $689.5^{\circ}$ ; pupation period,  $42^{\circ}$  to  $66.5^{\circ}$ . (The theoretical thresholds do not always indicate the lowest temperature at which progress can be made. For instance, the pupation period threshold is  $42^{\circ}$  F. and is satisfactory for most of the range but at a steady  $37.5^{\circ}$  many larvae will pupate and emerge in about 22 days. Again, the maturation threshold is  $32^{\circ}$  F., but only a small proportion of the flies will oviposit at  $41^{\circ}$  F. although they have been properly mated in the field. In spite of such deviations the application of the factors strictly has given good results.) By this standard the life cycles of *S. minima* have been timed for each week from 4th September, 1937, to those completing before 30th April, 1941, the process being fairly rapid once one gets used to the figures, as for the long larval period the data can be dealt with in blocks of summed daily means. These timed cycles were then superimposed on the charts of incidence and it was at once seen that the peak periods of output were caused by successions of generations running and alternating with one another.

Three such successions can be traced on the charts where they are designated by A, B and C, the letters against the appropriate peaks. The table gives the dates of the climaxes and shows the calculated date when the peak is due with the percentage error which may be on either side but is generally negative (twenty-eight cases out of thirty-five), the average error being 6.6 per cent.; eighteen fall within 7 days following the expected date and as each assessment covers a week these can be considered exact. Several short gaps when there was no trapping have had to be bridged. All temperatures are given in Fahrenheit scale as the average English reader thinks in this when dealing mainly with meteorological records.

Succession A has been followed from a bifid peak on 13th to 27th November, 1937. The bed on the former date registered about  $50^{\circ}$  and on the latter just over  $50^{\circ}$ , but in between the temperature fell smoothly to  $43^{\circ}$ , delaying pupal progress and rose again smoothly, and thus the saddleback was formed. At the end of the cycle in March the temperature was rising steadily so the offspring from 27th November gain a few days and the split closes to give a smooth peak

TABLE I.

PEAK PERIODICITY OF *S. minima* AND THEORETICAL TIMING PROVING THE SUCCESSION OF GENERATIONS.

Dates of Peaks.	Theory.	Duration in Days.	Percentage Error.
<i>Succession A.</i>			
Begins in a bifid peak.			
1937. 13. xi. } to 19. iii	13. xi. to 4. iii.	111	-11.9
27. xi. } to 19. iii	27. xi. to 14. iii.	107	-4.5
1938. 19. iii. to 28. v.	19. iii. to 27. v.	69	-1.4
28. v. to 23. vii.	28. v. to 15. vii.	48	-14.3
23. vii. to 3. ix.	23. vii. to 30. viii.	38	-9.5
3. ix. to 22. x.	3. ix. to 18. x.	45	-8.2
22. x. to 3. ii.	22. x. to 26. i.	96	-7.7
1939. 3. ii. to 12. v.	3. ii. to 3. v.	89	-9.2
12. v. to 30. vi. (?)	12. v. to 29. vi.	48	-2.0
30. vi. to 11-18. viii.	30. vi. to 10. viii.	41	-9.6
18. viii. to 29. ix.	18. viii. to 26. ix.	42	-7.1
29. ix. to 8. xii.	29. ix. to 13. xii.	75	+7.1
(a split occurs to 10. i. but rejoins)			
8. xii. } to 15. v.	8. xii. to 2. v.	145	-8.2
1940. 10. i. } to 15. v.	10. i. to 17. v.	128	+1.6
15. v. to 26. vi.	15. v. to 25. vi.	41	-2.4
26. vi. } to 14-21. viii	26. vi. to 5. viii.	40	-23.9
10. vii. } to 14-21. viii.	10. vii. to 18. viii.	39	exact
(the peak is obscured in a high level but reforms)			
14. viii. to 2. x.	14. viii. to 1. x.	48	-2.0
21. viii. to 16. x.	21. viii. to 12. x.	52	-7.1
(the flat-topped peak has split)			
2. x. to 19. iii. 1941	2. x. to 17. iii.	166	-1.2
16. x. to 2. iv.	16. x. to 12. iv.	188	+5.6
(the split has persisted)			
<i>Succession B.</i>			
1937. 23. x. to 29. i.	23. x. to 21. i.	90	-8.2
1938. 29. i. to 23. iv.	29. i. to 24. iv.	85	+1.2
23. iv. to 25. vi.	23. iv. to 20. vi.	58	-7.9
25. vi. to (no record)	25. vi. to 6. viii.	42	
(6. viii.) to 24. ix.	6. viii. to 16. ix.	41	
24. ix. to 19. xi.	24. ix. to 15. xi.	52	-7.1
(splits and second peak persists as Succession C)			
19. xi. to 24. iii.	19. xi. to 16. iii.	117	-6.4

TABLE II—(continued).

Dates of Peaks.	Theory.	Duration in Days.	Percentage Error.
1939. 24. iii. to 26. v. 26. v. to (no record) (gaps prevent further tracing)	24. iii. to 29. v. 26. v. to 10. vii.	66 45	+4.8
<i>Succession C.</i>			
1938. 17. xii. to 14. iv.	17. xii. to 13. iv.	117	—0.8
1939. 14. iv. to 16. vi.	14. iv. to 9. vi.	56	—11.1
16. vi. to (no record)	16. vi. to 29. vii.	43	
No record	28. vii. to 4. ix.	38	
No record to 3. xi.	8. ix. to 7. xi	60	
	3 generations	144	+2.1
3. xi. to 27. iii.	3. xi. to 20. iii.	138	—4.8
1940. 27. iii. to 5. vi.	27. iii. to 1. vi.	66	—5.7
5. vi. to 24. vii.	5. vi. to 14. vii.	39	—20.4
24. vii. to 4. ix.	24. vii. to 2. ix.	40	—4.8
4. ix. to 6. xi.	4. ix. to 3. xi.	60	—4.8

with an apex on 19th March. The succession then runs to 8th December, 1939, and a split occurs giving rise to a small peak on 10th January. This split also coincides with a temperature fall and the steep decline from 10th January indicates a time when the beds were snowbound and the jars could not be changed. This winter was so severe that days mild enough for mating occurred only on 1st, 2nd and 10th December, with no other till 27th February and any offspring from pairings between 24th November and 28th February would in theory emerge between 22nd April and 22nd May, the period bridged by the prominent peak whose apex is on 15th May, 1940. Thus the split is obliterated. The next peak in A is due on 25th June and may just emerge from a high level period which leads in turn to the flat-topped peak of 14th to 21st August. This is an interesting point as the peak splits when next evident and the split is maintained. Up to 19th August the mean bed temperature ranged over 60° and then fell steadily to 56.5° on 24th August with a following rise to over 60° on 27th August with high temperatures maintained till 10th September. The result was that when eggs from flies emerging on 21st August were due to hatch the larvae from those of 14th August had already accumulated 166 day-degrees and those of 21st August had to make up the deficit in the relatively cooler autumn days and a saddleback peak resulted on 2nd, 9th and 16th October. These flies encountered no important temperature fluctuation at the start of the next cycle and in the spring of 1941 two small peaks developed on 19th March and 2nd April. The former of these and the

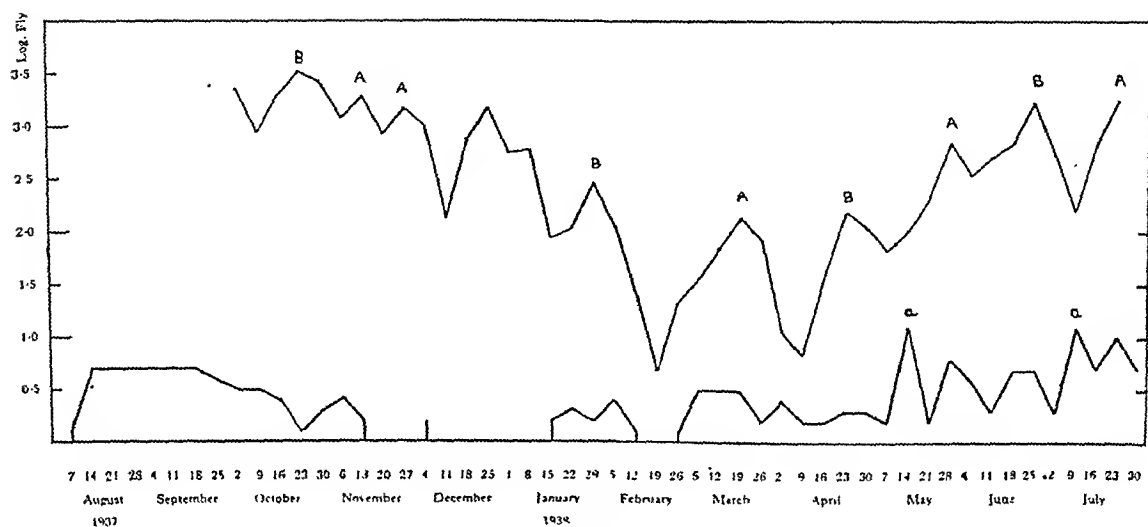


FIG. 1.

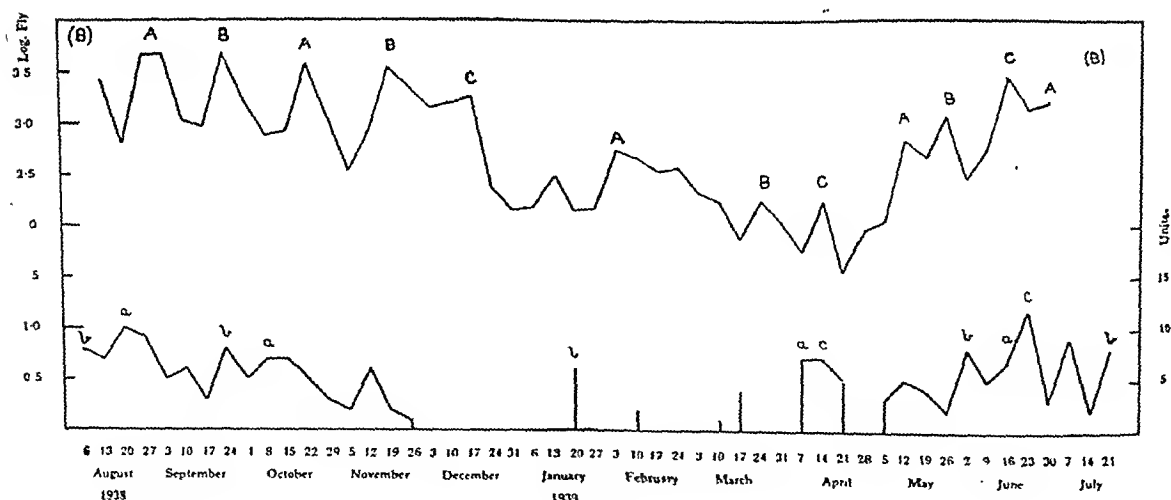


FIG. 2.

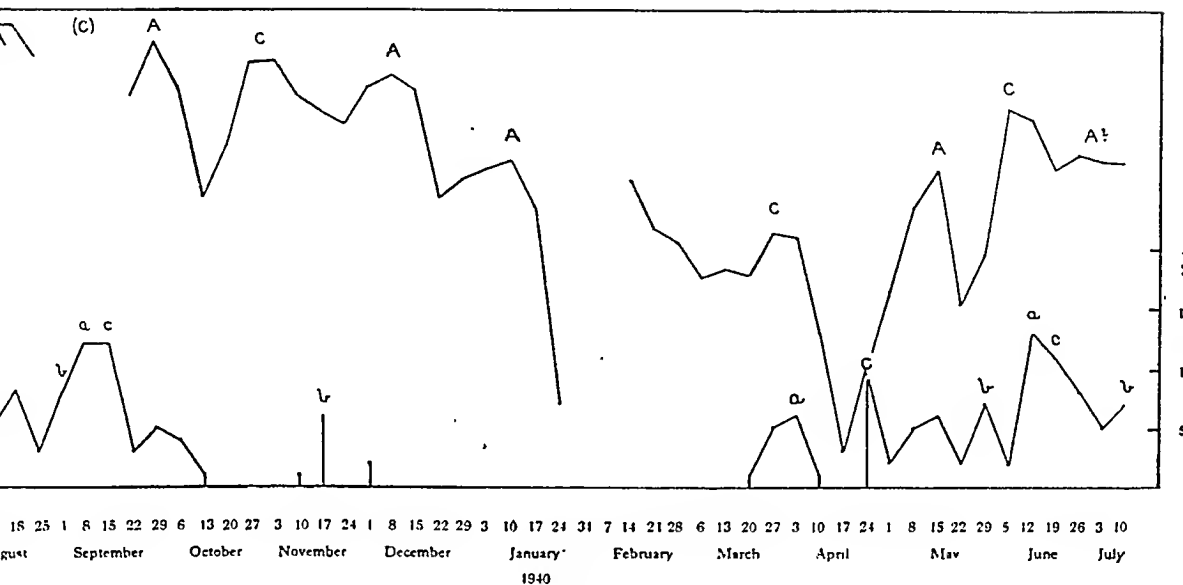


FIG. 3.

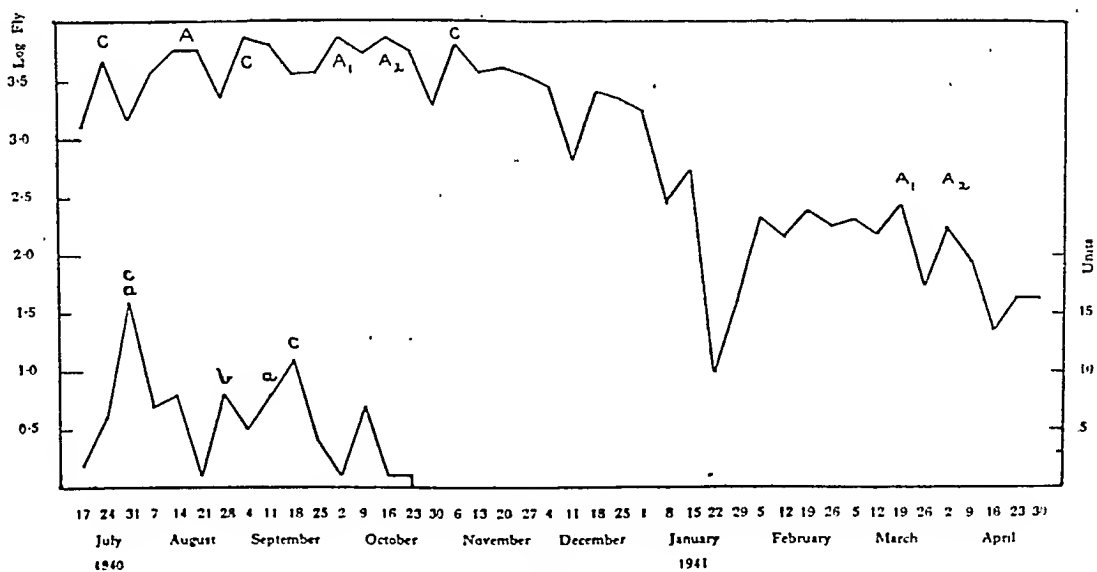


FIG. 4.

depression between them correspond almost to a day with what theory demands, but the peak of 2nd April is 10 days before expected. If this case stood alone no weight could be attached to it but in view of the theoretical case explained below and in conformity with other findings the formation of this part of the curve in spring is probably a true reflection of events in the previous August when the flat-topped peak split. The three sharp dips in the curve in this winter on 11th December, 8th January and 22nd January respectively were each associated with distinct temperature depressions but the peaks so outlined could not persist owing to the general severity of the winter. Thus A has been traced through eighteen appearances with only one doubtful point, 26th June to 10th July, 1940.

Succession B is readily followed from 23rd October, 1937, to 19th November, 1938, where it becomes saddleback owing to a temperature fall, the secondary peak on 17th December being the starting point of the third succession C. The depression between these two on 3rd December is reflected to a day and intensified on 7th April, and again reflected and intensified on 2nd June, 3 days before theory expects it. Thus succession B maintains itself through eight generations (with one gap bridged) till 26th May, 1939, having given rise to C, but thereafter cannot be traced and its fate is uncertain owing to two gaps in the record covering periods when it is due, 10th July and 10th August, 1939. Only two successions can be found thereafter.

Succession C has its origin as indicated on 17th December, 1938, and is readily traced to the gaps in the following summer. If these are bridged twice it is due again on 7th November and a well defined flat-topped peak is found on 27th October to 3rd November. No special temperature variations occur to split this peak and the succession persists through 1940 and is still distinct on 3rd November that year, its next appearance not being due when it was decided to close the tracing. It can thus be traced through ten generations and nearly two years if the two gaps in the record may be bridged by theory.

The precision with which these successions can be traced is evidence that *S. minima* in nature fulfils the requirements of the thermal constants as determined by laboratory breeding. The response is an average reaction and there is always some scattering in the emergence of a family with the same hatching date. Also the males are slightly quicker than the females on the average. Thus in seven families bred at 50° the offspring totalled 681 flies with an average cycle of 79 days and a scatter from 52 to 126 days; in nine families bred at 63° the offspring totalled 577 flies with an average cycle of 40 days and a scatter of 27 to 65 days. Such behaviour would tend to give long peaks in winter and spring, more abrupt ones in summer and autumn. There are, however, certain considerations which would lead one to expect a more steady seasonal output than the records yield. The breeding area is very great and flight from bed to bed occurs. The supply of food is steady and constantly renewed. Flies are emerging the year round and from April to October most

days are suitable for mating, November and March are often favourable and in a normal winter occasional days give the requisite temperature of  $50^{\circ}$  or over. About 150 to 200 eggs are laid and their hatching rate is nearly 100 per cent. The insect is a rapid breeder with between five and six cycles in the year. These considerations and the scatter in the families might be expected to suppress the tendency to peak outputs. It is, however, possible to show by a theoretical case the manner in which this type of incidence is imperative. This has already been briefly described (LLOYD, 1941).

It was supposed that there was a uniform population of one unit a day from 6th August to 21st September, 1937, and that the temperature of its environment was the mean shade temperature of the locality in order to obtain a quicker result than the bed conditions would afford. The life cycles were then traced by the thermal constants. Offspring from flies emerging on 6th August are due on 22nd September so a complete generation is covered by the forty-six units. No multiplication is required in the theory and they remain units throughout. Because of falling temperature the first complete generation occupies 164 days, the first family requiring 47 days for the cycle and the last family 118 (22nd September to 4th March). The next requires 83 days to 25th May, the next 51 days to 14th July, the next 39 days to 21st August. The temperature then begins to fall and the next cycle requires 55 days. The following cycle comes into the winter which, however, does not involve all the units, and it extends from 14th October, 1938, to 13th April, 1939, 182 days. Expansion and contraction are not steady processes owing to temperature fluctuations. The former leads to irregular scattering and the latter to clustering so that a number of units fall into one day. As mating can only occur in daylight these clusters become fixed in the system. Thus in the first cycle two units fall on 3rd February and in the next cycle three others join them on 14th May while a sixth is added on 7th July, 1938. When the population is assessed on a weekly basis these six units form the core of a peak as adjacent units or other clusters are added to them by chance before or behind. In the artificial system of course the peak is maintained indefinitely though in winter it might be confined to the six units. The curve of incidence yielded by this system is set out in the charts parallel with the curve of natural incidence and the close resemblance between the two is at once apparent. Three successions are recognised and marked *a*, *b* and *c*. The first, *a*, is based on the core of six units mentioned above. The second, *b*, is based on two pairs of units which join on 4th August, 1938, with a fifth added on 28th May, 1939, and a sixth on 16th June, 1939. The third, *c*, is formed by the overlapping of the first and last units of the system and the junction was overlooked in the first calculation so the figures here presented differ slightly from those of the earlier account. In the sixth cycle the last four units fall on 13th April, 1938, and the first unit of the seventh cycle falls on the same day. Other units are later absorbed till on 14th June, 1940, a core of nine units is formed through the overlap of the



eleventh and twelfth cycles. Core *a* and core *c* are separated by only one unit and together form the foundation of the very prominent peak that runs in the later part of the system. They fall into the same week twice, on 4th July, 1939, and on 31st July, 1940. The assessment on a weekly basis, of course, introduces a big element of chance into the elevation of the peaks. The argument is the same if the population is considered on a daily basis but the set out of this is too long for reproduction. It seems likely that if the calculations were carried on the original forty-six units would group themselves into two or three clusters.

### DISCUSSION.

The artificial system thus set up explains the fundamental meaning of the type of seasonal incidence that the insect displays. Sudden definitive rises in spring temperature tend to cluster the offspring of parents whose emergence was widely separated in time. Such a cluster in its succession forms the core of a peak with elevation dependent on seasonal increase or decrease. One can imagine these cores as isolated columns composed of individuals which adhere strictly to the average and overlaid by a layer of others whose cycles have been more hurried or retarded. The peaks thus overlap in their bases giving the characteristic seasonal level. Such a peak should be maintained indefinitely until it encounters an important temperature fall extending at least over a few days near the beginning or near the end of the cycle, fluctuations in the middle affecting the whole peak equally. Temperature falls in the middle of the cycle would tend to flatten the peak and a rise at any time would tend to steepen it. If it encounters a few days of low temperature near the beginning of the cycle then a split results. If at the end of this cycle the temperatures are fairly uniform, then the split is maintained and a daughter peak results. If, on the other hand, temperature is rising sufficiently near the end of the cycle, the split closes up as the later individuals have their development accelerated. Similarly, a split can form if a temperature fall occurs near the end of the cycle when the individuals are in or near pupation. These splits are particularly liable to form in early autumn and to persist because another fall is likely to involve the initiation of the next cycle. The first section may mature quickly but the second may be overtaken by the onset of winter.

In many insects the seasonal rhythm is regulated by the diapause which each year brings the species into activity at a time when weather and other conditions should be suitable. Others are regulated much less efficiently by the thermal thresholds and constants of the pupa state. For instance, amongst the sewage flies *Psychoda severini* has a pupal threshold of 34.5° F. and is efficient in winter except under very severe conditions. *P. alternata* has a pupal threshold of 46° F. and under natural conditions is distinctly a summer insect. *S. minima* has a pupal threshold in theory of 42° F. though, as mentioned,

pupation and emergence is possible at 37.5° F. Thus in weather of varying degrees of severity the pupal development of each of these flies is retarded and a certain probable limit is set to the climatic conditions that the adults have to face. Yet unseasonable spells of mild conditions can bring them out at awkward times. Thus in the theoretical case developed for *S. minima* the cluster of units *b* would have emerged on 20th January, 1939, in mild weather but not sufficiently so to give a suitable mating day till 20 days after emergence. In the artificial beds, of course, emergence is very frequent under conditions when successful mating is most unlikely.

This leads to a consideration of parallel behaviour in the seasonal rhythm of insects, particularly well known in Lepidoptera, a phenomenon of undoubted utility but of most obscure origin. A total emergence occurs in the earlier part of the season, and this is followed by a partial second generation, some individuals going into diapause with no apparent stimulus and so persisting till the following year. Thus a safety reserve is afforded should some weather disaster overtake the attempt at the second brood. *Hadena oleracea*, a tomato pest, is an excellent instance (LLOYD, 1920). Sometimes the safety reserve may persist in diapause through several years as in the puss moth (SHARP, 1901). In such ways is continuity ensured for the local race of a species.

In *S. minima* and many allied insects a critical phase is the mating dance without which copulation does not occur. Mr. N. H. E. GIBSON has recently made observations on the conditions governing this and confirms that an air temperature of 50° F. is necessary and finds that a very light wind prevents the dance. The thermal requirements do not prevent the fly from emergence in milder spells in the cold months and it might well be that if all were equally developed and timed in their cycle emergence in a stormy spell would prevent continuity. When the emergence of a generation is divided into a series of peak periods separated by an interval of two or three weeks such a disaster is not likely to overtake the whole local race. It seems a possibility that parallel behaviour in seasonal rhythm may have had its distant origin in the phenomenon here described and wholly dependent on physico-chemical reactions.

Finally, it seems imperative that all rapidly breeding insects whose seasonal rhythm is not governed by a diapause must react to temperature fluctuations more or less in the manner of *S. minima*. *Psychoda alternata*, of which an equally long record is available, certainly does so and its case is being considered. Only systematic collecting over a long period could reveal it, but the realisation that an insect of economic importance may be behaving in this way is worth bearing in mind. Especially should this be so where methods of control which ought to be successful break down.

The writer is grateful to the Management of Leeds Corporation Sewage Works, where these records have been collected, especially to Mr. J. T. THOMPSON, without whose generous help the records could never have been made.

It is not possible to publish the long details of trapping and temperature records nor the bulky calculations that the preparation of this paper has entailed. Copies of these and of certain charts that support the theory will be filed in due course at the University of Leeds and at Manson House, London.

#### SUMMARY.

The seasonal incidence of *Spaniotoma minima* (Chironomidae) is dominated by a series of very persistent peak periods which are due to successions of generations running and alternating with one another. A theoretical case is developed which proves that the insect must behave in this way. The phenomenon is probably common in rapidly breeding insects.

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## A STUDY OF THE CRUDE BIRTH/DEATH RATIO (VITAL INDEX) IN BRITISH GUIANA.\*

BY

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### INTRODUCTION.

Circumstances recently made it necessary to undertake a rapid survey of the vital statistics of the colony since 1900. The mass of raw data involved, the defects in registration of specific causes of deaths and the fact that the labour-saving equipment of modern biometrics were not available, directed attention to the usefulness of the Crude Vital Index.† It is realised that any operation which may suggest a "short cut" in the study of vital statistics may be suspected of fundamental inaccuracy. In view of PEARL'S (1923) commendation of the Vital Index, it was considered that an attempt was justified to ascertain some of the factors that might be determined from this ratio in British Guiana.

From the volume of data reviewed in annual reports it is not always possible to distinguish facts from impressions. Enthusiasm for certain aspects of public health effort sometimes tends to overshadow other equally important features ;

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† Crude Vital Index =  $\frac{100 \text{ (registered births)}}{\text{deaths from all causes.}}$

furthermore, the annual survey which is concerned with whether the year under review showed better or worse results than the previous year, may for that reason fail to give due emphasis to trends. In approaching this study, a newcomer to the colony is faced with the following questions: what is the

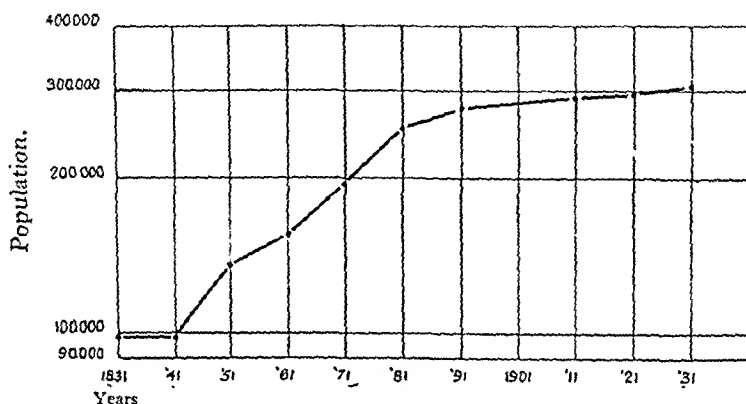


FIG. 1.—Census Population, British Guiana—1831-1931.

major public health problem, what group does it most affect, and where is that problem most serious. This paper purports to show the line of inquiry and some of the information obtained. The study is not exhaustive and may suffer from *a priori* reasoning as well as some cardinal errors that a professional statistician would avoid, but it is thought that record may have justification.

TABLE I.

POPULATION DATA—BRITISH GUIANA

Years	Decennial Census Populations	Net Increase	Natural Increase*	Calculated Change of Population by Migration
1831	98,000	—	—	—
1841	98,154	154	—	—
1851	135,994	37,840	—	—
1861	155,907	19,913	—	—
1871	193,491	37,584	—	—
1881	252,186	58,695	+ 3,532	+55,163
1891	278,328	26,142	+ 1,323	+24,819
1901	296,041	17,713	+ 916	+16,797
1911				
1921	297,691	1,650	— 8,544	+10,194
1931	310,933	3,342	+18,907	—15,565

(\* For decenniums—Total registered births less total registered deaths).

Vital statistics have been collected in British Guiana since 1869 and, although the conventional classifications of causes of deaths have altered much over that period, the records of births and deaths are not subject to such alterations. Decennial population census determinations have been recorded (except in 1901) since 1831, the curve (Fig. 1) of which shows the effects produced by the introduction of indentured immigrants. The tabulated data show certain interesting population changes upon which comment is unnecessary in this paper. It is not profitable to introduce the history of this experience with

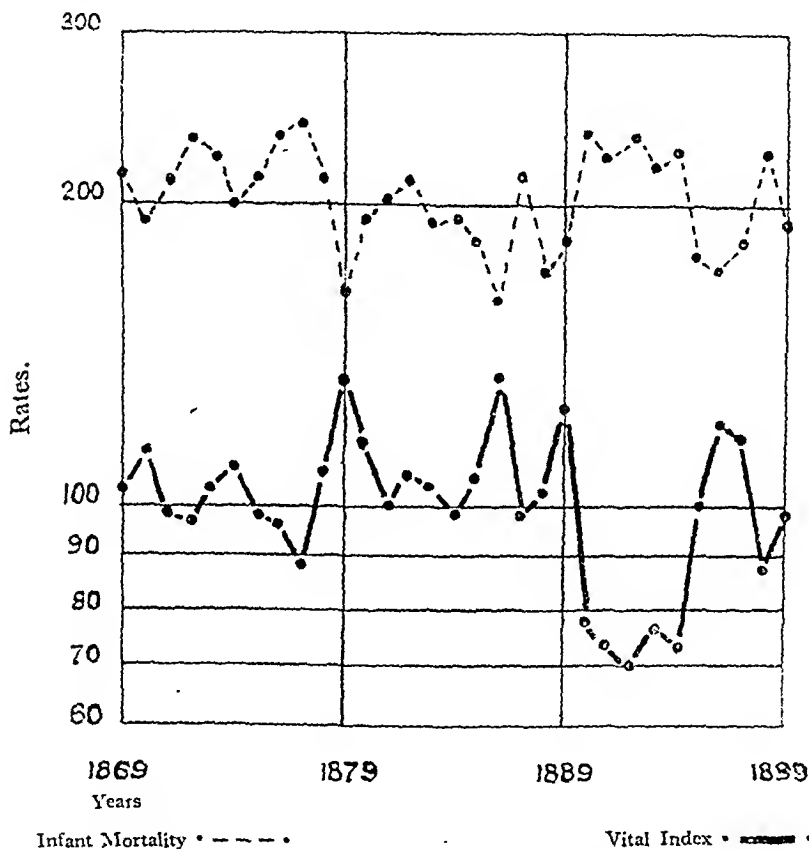


FIG. 2.—Crude Vital Index and Infant Mortality Rate—1869-1899.

indenture other than to record that it explains the variety of races represented in the present population, that it ceased in 1920, and that repatriation since that time has resulted in an insignificant depletion of the colony's population.

The crude vital indices and infant mortality rates from 1869 to 1900 projected in Fig. 2 serve as an interesting comparison with those of the present century. While it does not follow that the deductions drawn from contemporary events are necessarily applicable to the past, it is probable that they are not totally dissimilar. Whatever may have been the specific contributory reasons,

the vital index curve over that period indicates a remarkably low rate of natural increase, characterized by extended periods indicative of adverse conditions.

TABLE II.

CRUDE VITAL INDEX AND INFANT MORTALITY RATE—BRITISH GUIANA, 1869-1899.

Year	Births No.	Deaths No.	Crude Vital Index	Infant Mortality Rate per 1,000 Births.
1869	6,664	6,334	105	216
1870	7,103	6,203	114	192
1871	7,182	7,324	98	214
1872	7,032	7,354	96	233
1873	7,558	7,245	104	222
1874	8,317	7,567	110	200
1875	7,825	8,034	97	215
1876	7,833	8,270	95	235
1877	7,585	8,677	87	241
1878	7,960	7,282	109	211
1879	9,211	6,735	137	168
1880	8,975	7,649	117	196
1881	8,144	8,095	100	204
1882	9,105	8,451	108	211
1883	8,172	7,867	104	191
1884	7,869	8,076	97	192
1885	8,551	7,973	107	187
1886	9,661	7,014	138	162
1887	8,949	8,979	99	213
1888	8,575	8,153	105	171
1889	10,183	7,937	128	183
1890	8,726	11,230	78	238
1891	7,422	10,210	73	227
1892	7,795	11,070	70	232
1893	7,593	9,880	77	220
1894	6,957	9,374	74	228
1895	8,177	8,345	100	180
1896	9,276	7,513	123	172
1897	9,635	8,000	120	184
1898	8,500	9,706	88	228
1899	8,275	8,352	99	191

#### SPECIFIC FACTORS.

The crude vital index from 1900 to 1938 (Fig. 3) shows a continuance of the unfavourable conditions until 1919, similar to those indicated in Fig. 2, since which time there has been a gradual improvement characterized by fairly regular recessions. The explanation of these recessive phases has been sought

by correlation with the specific mortality rates since 1900. An unrecorded epidemic which produced an unusually high mortality rate, classified as respiratory diseases in 1910 and epidemic influenza in 1918-1919, are both reflected in the vital indices of those years—the latter more noticeably. Study of the other specific causes of deaths indicates that the malaria and undefined fevers mortality rate curve and the infant mortality rate curve show the closest correlation with that of the vital index. These correlations are shown in Fig. 3. The fact that these curves represent three different mathematical universes may to some extent vitiate confidence in their relative significance.

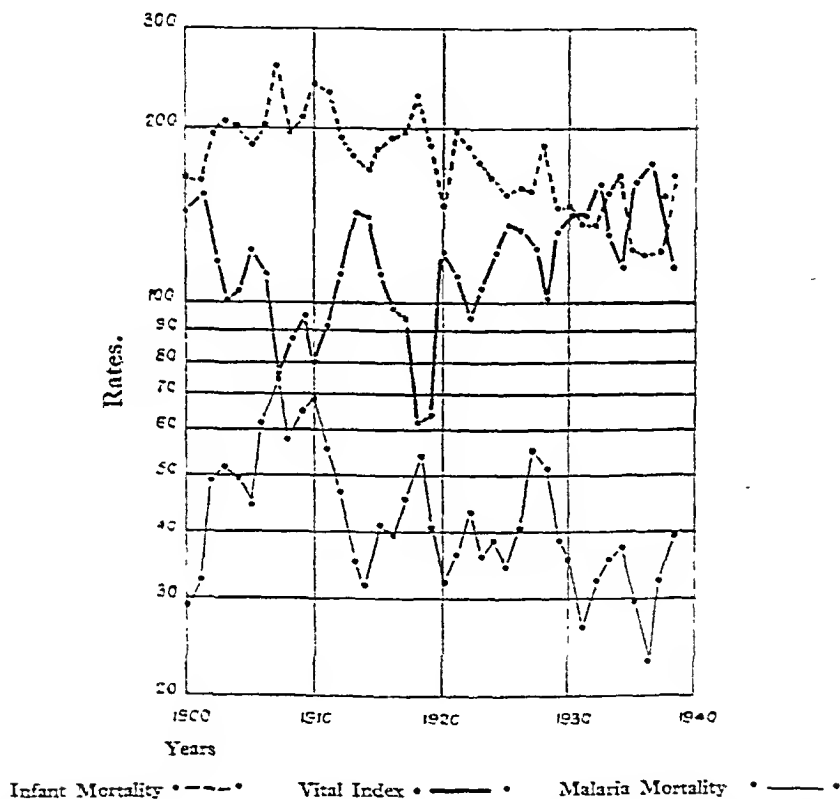


FIG. 3.—Crude Vital Index, Infant Mortality and Malaria Mortality Rates, 1900-1938.

For various reasons it has been considered that infant welfare activities have been chiefly responsible for the improvement in the infant mortality rate of the colony. In consequence, one's first impression was that the falling trend of this rate might explain the improvements in the vital index. Suspicion that this was not the case arose from the inverted peaks of 1922, 1928 and 1934. These suggested the epidemic incidence of some disease that was not necessarily restricted to early infancy. It is noteworthy that for some unexplainable reason malaria is seldom recorded in this colony as the cause of an infants' death.



TABLE III.

CRUDE VITAL INDEX. INFANT MORTALITY AND MALARIA MORTALITY RATES  
BRITISH GUIANA—1900-1938.

Year	Crude Vital Index	Infant Mortality Rate per 1,000 Births.	Mortality Rate, Malaria and Undefined Fevers per 10,000 Population.
1900	145	164	29.2
1901	152	162	32.4
1902	119	194	49.0
1903	100	205	51.7
1904	105	201	49.3
1905	124	187	44.1
1906	114	202	61.8
1907	76	256	75.9
1908	88	198	57.2
1909	96	209	64.8
1910	80	235	68.2
1911	91	229	55.6
1912	113	190	46.8
1913	143	179	35.9
1914	141	170	31.5
1915	113	184	40.5
1916	98	190	39.2
1917	94	199	45.7
1918	62	223	54.0
1919	64	185	40.5
1920	124	148	32.7
1921	112	195	36.8
1922	95	186	43.4
1923	107	177	36.1
1924	126	165	38.1
1925	139	155	34.6
1926	136	159	40.1
1927	125	158	55.6
1928	101	185	50.8
1929	135	146	38.7
1930	145	146	35.3
1931	144	139	26.6
1932	161	139	32.5
1933	133	154	35.5
1934	116	168	37.2
1935	166	122	29.1
1936	173	120	22.7
1937	152	121	32.3
1938	117	166	39.6

To test this aspect, the total infant deaths were deducted from both the birth and death factors entering into the calculations of the crude annual vital index. This adjusted vital index so computed flattened the curve insignificantly but did not alter the trend. Under these circumstances it seemed evident that malaria and undefined fevers must have been the major factors affecting the fluctuations of the crude annual vital index. Similarly, such improvement as is indicated by this index must be associated with either an absolute reduction in the incidence of malaria or the effects of premunition.

Lacking information as to the incidence of malaria, it has been assumed that there is a reasonably constant relationship between mortality rates and the incidence of this disease. It has not been possible to verify this from public hospital statistics, because there is evidence that until 1927 the in-patient treatment of malaria had ceased to have the importance in hospital practice that it had in the first decade of this century. With the acceptance of this assumption, reference to the curve suggests that since 1920 this colony has presumably suffered at intervals from malaria in epidemic form. It may not be valid from this to conclude that this alone explains the past experience shown by the vital index curve since 1869. It does, however, give reason for suspicion, and in view of contemporary knowledge of the immunological factors associated with malaria, it must naturally raise doubt as to the desirability of diluting resident tropical populations with large numbers of immigrants who are without tolerance to the local strains of plasmodia, unless special precautions are taken for their protection. Here at least is some suggestion of premunition developing in a population that is not now subject to immigration. Because of its relative geographical isolation, this population is now in the favourable position of being relatively free from exposure to the introduction of foreign strains of malarial plasmodia, and for that reason it is possible to postulate that premunition may be a factor of some importance.

The fact that malaria is endemic in British Guiana may explain the failure to appreciate the epidemic phases of the disease until recently. GIGLIOLI's investigations (1938) record the only extended studies upon the malaria problem in British Guiana. His use of the crude vital index has been restricted to the populations of selected groups of sugar estates. These groupings were made upon the basis of his observations of splenic indices, the prevalence of the major anopheline vector and the incidence of the environmental conditions favourable to that vector. The significance of endemic malaria as a dominant factor in the crude vital indices of these estates is thus reasonably established.

Since early in 1937 it has been possible to observe the development and distribution of malaria in epidemic form, to observe the effects of the epidemic upon the vital indices and to correlate these with the specific factors comprising the colony's vital statistics. From this experience it is possible to state that the adverse conditions indicated by the crude vital indices of 1937 and 1938 are chiefly reflective of the epidemic incidence of malaria. From this restricted

appears to be a general similarity in the curves concerned with Demerara and Essequibo, the conditions responsible for the experience in Berbice is similar only in so far as the years 1933-34 appear to indicate the possibility that a colony-wide epidemic occurred coincidental with the increased mortality rate of malaria during the years 1932-34. If experimentation on malaria control may be justified on a county-wide basis, it would appear that Berbice would be the choice for efforts directed towards the investigation of the epidemic factors and Demerara for the endemic problems.

#### RACE VARIATIONS.

Race as a factor in epidemiology does not often present such striking differences as are shown in Fig. 5. The populations of the different races

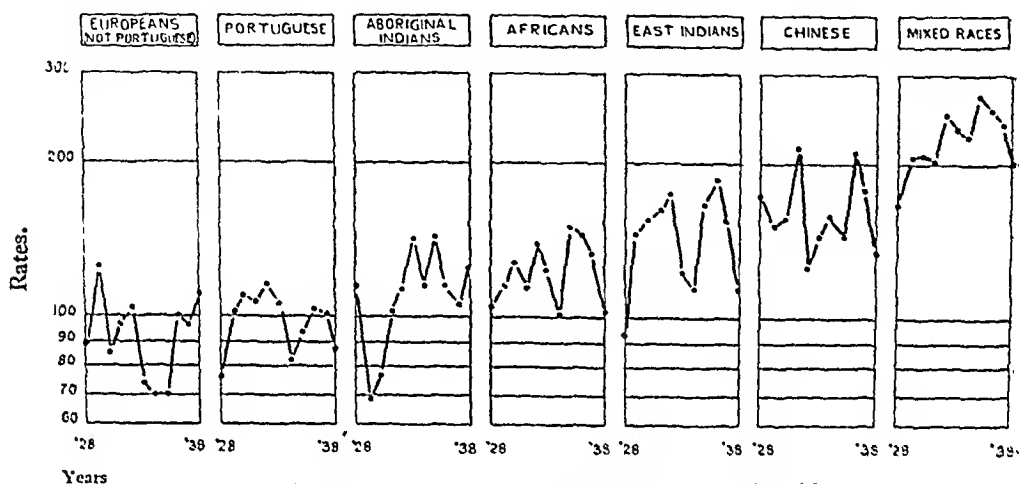


FIG. 5.—Crude Vital Index : By Race 1928-1938.

represented differ materially, and so far as the curve for Europeans (not Portuguese) is concerned, requires some explanation. This group is not fairly represented because there may be a tendency for women of this group to return to their country of origin for confinement, for persons of the older age groups to retire from the country and for those who are ill to withdraw. For these reasons births and deaths in this group probably are not indicative of the true facts. Statistical information about the aboriginal Indians is notoriously inadequate and the trends here shown are indicative of conditions affecting a fractional proportion of this group who are settled only within the immediate knowledge of the District Registrar concerned. Furthermore, there is some reason to question whether the designation of this as a pure racial entity is not loosely applied. The other racial groups, despite population disparity, have reasonably precise recognition.

TABLE V.  
BRITISH GUIANA—VITAL INDEX. BY RACES, 1928-1938.

Races.	Years	1928.	1929.	1930.	1931.	1932.	1933.	1934.	1935.	1936.	1937.	1938.	Census Population, 1931.
European (not Portuguese)	Births	33	37	30	31	30	27	23	23	25	28	29	2,127
	Deaths	37	29	36	32	29	37	33	33	25	29	26	
	V. Index	89	128	83	97	103	73	70	70	100	97	111	
Portuguese	Births	188	181	180	169	179	189	152	167	168	185	179	8,612
	Deaths	251	175	164	156	151	173	185	180	164	183	207	
	V. Index	75	103	110	108	118	109	82	93	102	101	86	
Aboriginal Indian	Births	252	211	257	296	321	406	304	374	364	357	349	8,348
	Deaths	347	307	333	289	256	279	260	252	313	334	277	
	V. Index	115	69	77	102	125	145	117	148	116	107	126	
African	Births	3,255	3,419	3,464	3,137	3,545	3,565	3,221	3,746	3,697	3,675	3,292	124,203
	Deaths	3,076	2,903	2,672	2,680	2,521	2,823	3,137	2,481	2,473	2,696	3,238	
	V. Index	106	118	130	117	141	126	103	151	149	136	102	
East Indian	Births	3,850	4,666	5,201	4,978	5,441	4,942	4,244	5,452	6,088	5,544	4,881	
	Deaths	4,177	3,183	3,304	3,073	3,184	3,947	3,729	3,240	3,277	3,503	4,287	130,540
	V. Index	92	147	157	162	171	125	114	168	186	158	114	
Chinese	Births	79	65	71	61	57	59	61	62	63	73	55	2,951
	Deaths	46	43	45	28	44	41	38	43	30	41	41	
	V. Index	172	151	158	218	129	144	160	144	210	178	134	
Mixed Race	Births	1,043	1,240	1,233	1,180	1,250	1,270	1,296	1,438	1,328	1,365	1,231	33,800
	Deaths	621	615	605	580	500	537	568	517	514	570	617	
	V. Index	168	202	204	203	250	236	238	278	258	239	199	

The significance of the array of these racial groups offers some indication of the extent to which the imported races have been able to accommodate themselves to the conditions prevailing in the colony. Undoubtedly the situation indicated with respect to the Portuguese is serious. If malaria is the major factor, it appears that this race shows no evidence of having acquired a tolerance to its effects. The populations of Africans and East Indians are the most nearly comparable in numbers and despite the fact that, as a race, the former have been established here the longer time, it seems clear that the rate of natural increase of the East Indian population exceeds that of the persons of African origin. On the other hand, the nature of the 1933-34 decline, presumably arising from epidemic malaria, suggests that the East Indian population was affected more promptly and more seriously. Whether this may be due to race or racial habits alone or may be connected with the East Indians' predominant association with sugar estates and rice cultivation, and all that these may imply from exposure to the vectors, is outside the range of these deductions. The total Chinese population is comparatively small, and for that reason the fluctuations of the index may have little real significance, but the general trend gives some indication that this racial group has accommodated itself to the prevailing conditions to a more satisfactory extent than any of the other distinctive racial groups. The most striking feature of the array is to be seen in the group designated Mixed Races. It is not possible to know the degree or variety of racial admixture represented in this group. The well-known observation as to the hardihood of hybrids seems substantiated in this particular curve and suggests the probability that in time this group may predominate in British Guiana. The effect of the 1933-34 epidemic was minimal, but the 1937-38 epidemic seems to have had a more adverse result upon the natural increase of this group. If a geneticist with an immunological bias might be able to unravel the mystery of the apparent resistance of this group to the effects of endemic malaria, it may be possible to contemplate another method of meeting the problems created by that disease.

#### COMMENT.

It is realised that the vital index must be the result of various contributory factors of a sociological and economic nature as well as those factors that are immediately concerned with the specific causes of morbidity and mortality. From the existing information it has not been possible to differentiate these, but analysis of these other problems should provide an interesting subject of study. This particular inquiry has been undertaken with the object of determining to what practical epidemiological use this ratio may be applied in a colony where provision has not been made for serious use of the biometrical data which have been collected for a number of years. The average person is not concerned with specific mortality rates and statistical reports from medical

departments and general registry offices make very poor newspaper "copy." The same average person usually does possess sufficient sense of "profit and loss" to understand the significance of the rate of natural increase in a population. The vital index thus appears to meet this sense of value to such an extent that it would seem to warrant wider usage in presenting facts that concern the health of the public.

It is submitted that the vital index curves here studied provide a useful and convenient indication of the relative importance and location of epidemiological problems under the conditions that obtain in British Guiana. In the event that major malaria control measures prove to be practicable, there is reason to believe that an indication of the effectiveness of these may be followed by continued observation of these ratios. It seems evident that due recognition must be given to the endemic and epidemic distribution of the colony's predominant disease, malaria; furthermore, there appears to be a possibility that premunition explains the conspicuous improvement of the crude vital index since 1919, in which race appears to have an important rôle.

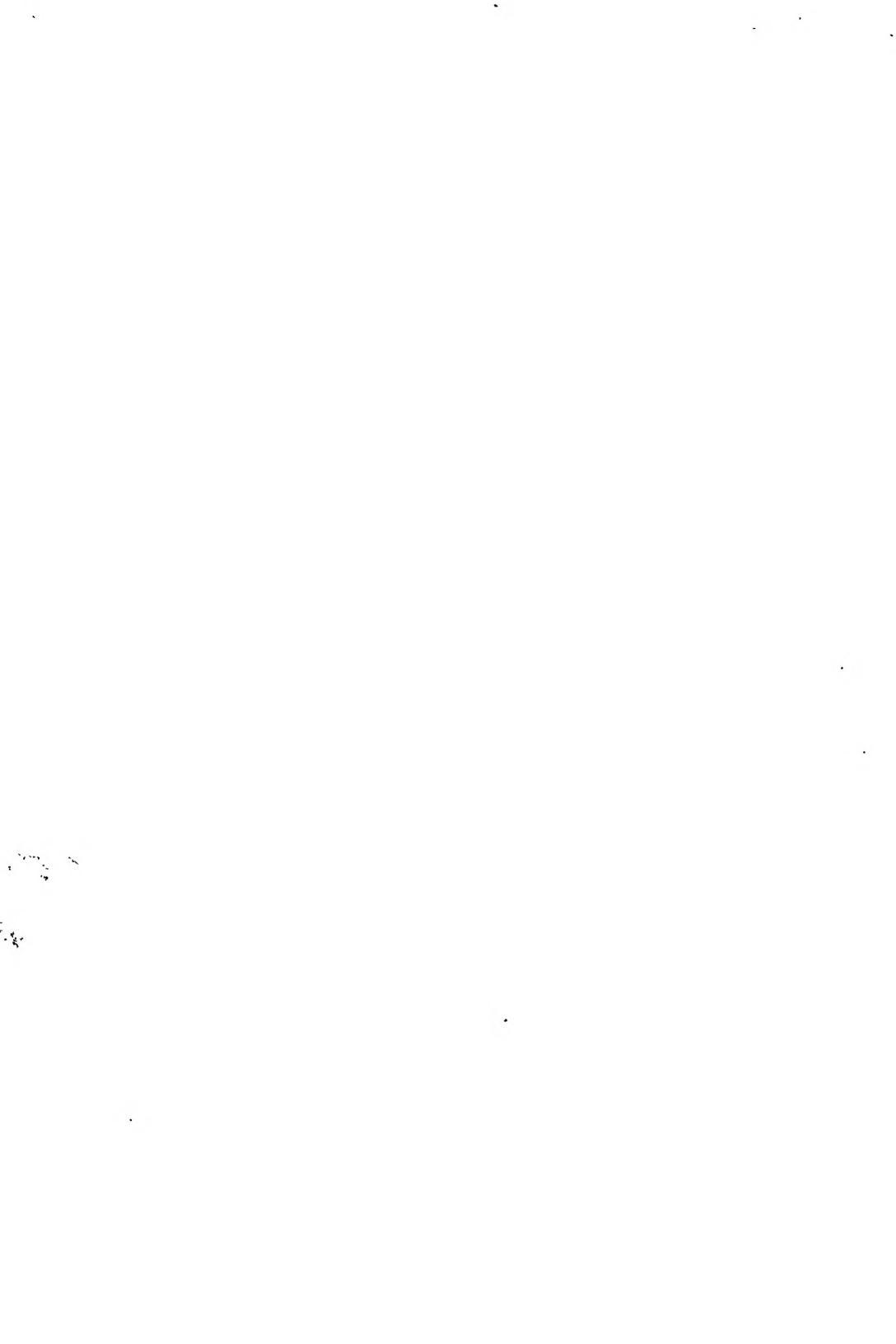
#### SUMMARY.

1. A study of the crude vital index of British Guiana is presented. From this there is evidence that malaria is the dominant factor in the birth/death ratio and that this disease recurs in periodic epidemics. The conditions reflected by this index show definite improvement since 1919. It is suggested that as a result of the cessation of immigration, the improving conditions indicated by the rising trend in the vital index curve and the falling trend in the malaria mortality rate, there may be evidence of the development in the population of premunition to the prevailing strains of malaria.

2. With the foregoing in mind, the crude vital index curves for locality and race afford a convenient means of ascertaining the effect of the colony's major health problem with regard to these specific factors.

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## TRANSFUSION OF BLACKWATER FEVER BLOOD INTO A NORMAL INDIVIDUAL DURING HAEMOLYTIC CRISIS.

BY

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In a previous paper we described (FOY & KONDI, 1936) a series of injections of blackwater fever blood, taken after varying intervals from the first passage of black urine, into lunatics. These bloods were all injected intramuscularly. In some cases the lunatics developed malaria (*P. vivax* or *falciparum*), although the blackwater fever blood injected was negative for malaria (thick film); in other cases a positive blackwater fever blood gave a positive result in the lunatic. In all 106 lunatics were injected from fifty-eight cases of blackwater fever. The lunatics were observed for from 9 to 18 months, but in not a single instance did blackwater fever develop.

From these experiments we concluded that the field of discussion on the existence of haemolytic strains of malaria and/or other specific parasites was narrowed down.



There remained, however, certain other points which these experiments did not clear up, particularly the point as to whether blackwater fever patients possessed red blood cells peculiarly liable to haemolysis by specific auto-haemolysins; and whether blackwater fever patients could haemolyse cells of normal individuals if transfused into them. The present paper is an attempt to answer both these questions by means of direct transfusions during haemolytic crises. Our experiments not only covered transfusion into blackwater fever patients of normal blood, but also transfusion of blackwater fever blood into normal individuals.

Below we outline our first case; we hope later to follow this up with more work in greater detail.

#### CASE.

*On 24th September, 1940*, a woman, aged 24 years, entered our wards with blackwater fever. On entrance, she was passing thick, black urine containing oxyhaemoglobin and methaemoglobin. She gave no previous personal or family history of blackwater fever. She stated that on 22nd September she felt "out of sorts" and went to a doctor and was given an intramuscular injection of quinine bihydrochloride at noon; at midnight she passed her first lot of black urine. All through the 23rd she continued to pass black urine. She entered our wards on 24th September, very icteric, spleen I (Hackett) soft, liver enlarged to three fingers, and very tender. She was conscious, but obviously very gravely ill. Her urine on entrance was black, acid, and contained oxyhaemoglobin and methaemoglobin.

*On 25th September*, Laboratory examination:—

Red blood cells: 1,520,000. ( $\pm 3$  per cent.)

Bilirubin: direct ++, indirect 8 mg. per cent.

Reticulocytes: 1 per cent.

Methaemalbumin (spectroscopic dilution factor): 1 in 2.

Oxyhaemoglobin: 80 mg. per cent.

Alkali reserve: 32.3 c.c.  $\text{CO}_2$ .

Parasites: negative and no malaria pigment.

During the 25th the patient passed 2,100 c.c. of black, acid urine containing oxyhaemoglobin and methaemoglobin.

*On 26th September* the patient was much worse, vomiting, comatose and defaecating in the bed. The red blood cell count had fallen to 756,000 ( $\pm 3$  per cent.) and the urine collected by catheter was copious, black, acid and evil smelling, containing a few casts, pus cells and trichomonads. As the patient was still haemolysing and passing plenty of urine, showing that renal function was being maintained, we decided to transfuse her and she was given 300 c.c. of citrated whole blood at 11.30 a.m. by means of a Tzanck apparatus. By reversing the apparatus the donor was first given 25 c.c. of blood from the blackwater fever patient; the apparatus was then again reversed and the transfusion carried out without hitch. Three hours after this transfusion the patient's red cell count was 1,316,000 per c.mm., a rise of approximately 500,000.

27th September.—The patient continued to pass black urine in large quantities (2,700 c.c.), and 24 hours after the transfusion the red cell count was back to 1,028,000 ( $\pm 3$  per cent.).

28th September.—The red cell count had now dropped to 836,000 per c.mm., reticulocytes 1.6 per cent. As she was still passing a quantity of black urine another transfusion was given of 300 c.c. at 11.30 a.m. Three hours later her red blood cell count was 1,296,000.

29th and 30th September.—The haemolysis continued and she passed large amounts of black urine. On the 30th her R.B.C. count was again down to 840,000, reticulocytes 1 per cent., and a further transfusion of 300 c.c. was given at 11.30 a.m. on 30th September. Three hours after this transfusion her red cell count was 1,346,000.

During the following 4 days the urine gradually cleared, although on 4th October her blood count was down to 843,000; reticulocytes 15 per cent.

On 7th October her blood count was 1,200,000 and reticulocytes 26 per cent., and urine free of all trace of haemoglobin, but loaded with trichomonads.

The patient thereafter made a slow but uneventful recovery.

To return to the man who was given 25 c.c. of the blackwater fever patient's blood on September 26th. He was kept under close observation, and there was nothing to report until 5th October, when he went down with a smart attack of fever,  $45.5^{\circ}\text{C}$ ., his peripheral blood containing *P. falciparum* rings in the thick film. He was given 1 gramme of quinine bihydrochloride intramuscularly. On 6th October his temperature was down to  $37.8^{\circ}\text{C}$ . He was given 2 grammes of quinine again intramuscularly. His spleen was not palpable, nor was his liver enlarged or tender.

On 7th October his temperature was again up to  $39^{\circ}\text{C}$ ., and rings were still present in his peripheral blood. The quinine was continued until he had been given 8 grammes. He was then given atebrian, and made an uneventful recovery. The only point noted was that the parasites appeared to be very resistant to both quinine and atebrian, as the man was still running a fever with a few parasites in his blood after 8 grammes of quinine and 1 gramme of atebrian.

#### DISCUSSION.

Twenty-five c.c. of blood from a haemolysing case of blackwater was transfused into a normal healthy man with a previous history of malaria in 1930. Eleven days after the transfusion he went down with a smart attack of fever with *falciparum* rings in his peripheral blood. The transfusion of this blackwater fever blood failed to produce any sign of haemolysis, although the recipient went down with malignant malaria. There was no possibility of the man having become infected with malaria before his transfusion, nor could he have been infected afterwards, as we had him under our own observation in the hospital. There can be no doubt, therefore, that he developed his malaria as a result of his transfusion.

His failure to develop either blackwater fever or any other sign of haemolysis seems to us to cast serious doubt on the view that there is a haemolytic strain of malaria, or that there are other specific parasites (spirochaetes) involved in the genesis of blackwater fever (cf. FOY & KONDI, 1936).

At first sight it would appear that the absence of any sign of haemolysis in the man might have been due to the fact that the blackwater fever blood at the time of the transfusion contained no haemolysins. But this is discounted by the observation that the woman continued to haemolyse blood that was transfused into her on three successive occasions, as shown by the fact that her blood count continued its rapid fall after having been brought up on three occasions to 1.34 millions by transfusions. Further, her urine continued to be heavily laden with oxyhaemoglobin and methaemoglobin for many days after the first transfusion. It seems clear, then, that either the 25 c.c. of blood transfused into the man contained insufficient haemolysins to bring about a haemolysis in the healthy man, or if any haemolysin was transferred it was immediately dealt with.

A further point of interest is that the blackwater fever patient's blood possessed the power to destroy the red cells transfused into her from three different donors; thus making it evident that there is nothing peculiar about the red cells of blackwater fever patients that renders them particularly susceptible to haemolysis. We base our conclusions that all normal red cells are susceptible to haemolysis on the facts that on 26th September our blackwater fever patient's red cell count had fallen from 1.52 millions per c.mm. to 756,000 with reticulocytes at 1.4 per cent. At 11.30 a.m. on that day she was transfused with 300 c.c. of citrated whole blood and three hours later her red cell count was up to 1,316,000 per c.mm.\* Two days later, on 28th September, the patient's count had again fallen to 836,000, with reticulocytes at 1.6 per cent. A second transfusion was given of 300 c.c. blood, and three hours later the count was 1,296,000. On 30th September the woman's count had again fallen to 840,000, with reticulocytes at 1 per cent. She was given another transfusion on the 30th of 300 c.c. and her count rose to 1,346,000. On 4th October her count had again fallen to 843,000 but as her reticulocytes were now at 15 per cent. and she was passing clear urine we decided not to give her another transfusion. Since during the period when she was given her three transfusions her reticulocytes were never above 1.6 per cent., it looks as though her own blood regeneration was not active, and that the rises in her red cell count were due to the transfusions and the falls to the continuing haemolyses, establishing our point that the blackwater fever woman was not only able to haemolyse the red cells transfused into her, but also that her blood actually did contain haemolysins at the time we gave the 25 c.c. to our normal man.

In a previous paper (FOY & KONDI, 1941) we reported a case of blackwater fever in a pregnant woman who gave birth to a child during her attack of blackwater fever, when she was actively haemolysing; there was no sign of haemolysis in the child, as shown by the absence of icterus, and oxyhaemoglobin and methaemalbumin in the child's blood. We concluded from this that either

\*We have found that 300 c.c. of transfused blood is approximately equivalent to 400,000 red cells per c.mm. in the case of all three of these transfusions.

(1) the cells of the infant were not susceptible to the haemolysins produced by the mother, or (2) that the maternal haemolysins did not pass the placenta, or (3) that there were insufficient haemolysins to bring about haemolysis in both mother and child. From the present transfusion experiment either (2) or (3) would seem to be the explanation of the absence of haemolysis in the child and not that the cells of the infant were less susceptible to haemolysis than those of the mother.

#### SUMMARY.

From the transfusion of blackwater fever blood into a normal healthy adult with a previous history of malaria, it would appear that there are no specific parasites or haemolytic strains of malaria concerned in the genesis of blackwater fever, since the recipient of haemolysing blackwater fever blood failed to develop blackwater fever, or any other sign of haemolysis, although he went down with malignant tertian malaria 11 days after he received the blackwater fever blood. The possibility of his having been infected from other sources was absolutely excluded.

The fact that the man failed to show any sign of haemolysis, although haemolysins were present in the blood he received, may have been due to the fact that he received insufficient blood; or that he dealt with any haemolysin he received immediately.

Blood cells transfused from three normal individuals into a haemolysing case of blackwater fever underwent rapid haemolysis, showing that the red cells of normal individuals are just as susceptible to haemolysis when transfused into blackwater fever patients, as are the blackwater patient's own cells. This fact seems to us to dispose of the view sometimes put forward that the red blood cells of blackwater fever patients are more susceptible to haemolysis than are normal cells, and makes it appear probable that a circulating haemolysin may be responsible for the trouble. Of course it is by no means improbable that continuous sensitization is necessary before the red cells become liable to haemolysis, or infection over long periods with a special strain of malaria, and that one infection is not sufficient to sensitize the individual.

Transfusion, even in moribund cases with red cell counts as low as 800,000 per c.mm., is a life-saving measure, provided that renal function is being maintained.

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1939, when the toe became inflamed and required dressings, but he continued at work until admission to hospital. Dead skin used to come away from between the toes and a few months ago he noticed a dry cleft in the right toe.

*Examination* revealed a typical bilateral ainhum more advanced on the left side. There was a constricting band around the middle of the proximal phalanx of the left toe and a deep cleft surrounding all but a little of the dorsum was lined by hard dry skin. The distal part of the toe was swollen but not gangrenous. There was hyperkeratosis of the soles of both feet and of the skin around the nail beds of all the toes. Sensation was absent in the distal part of the left little toe but present in that of the right toe. No other changes were found in the nervous system. The blood pressure was 130/90 and the heart appeared healthy.

*Skiagrams* of the legs showed marked calcification of the tibial and dorsalis pedis arteries and the interosseous arteries but the pulse in the dorsalis pedis was of good volume on both sides. Radiologically the shaft of the proximal phalanx of the left toe showed considerable



FIG. 1.—Ainhum of left little toe.

thinning, due mainly to absorption of the cortex, and the head of the bone was largely absorbed. Some slight absorption was present in the proximal phalanx of the right toe. There was also some collapse of the transverse metatarsal arch. The Wassermann and Kahn reactions were negative and haematological investigations showed no abnormality.

Both little toes were amputated by Mr. H. CHITTY who noticed that there was much less bleeding from the stump of the left toe than from the right. Post-operative healing was slow. On walking he suffered from intermittent claudication which improved with massage. He returned to work in March, 1941.

#### *Histological Report.*

*Left Toe.*—A transverse section through the middle of the proximal phalanx just proximal to the constriction shows a very thick superficial layer of keratin on the plantar aspect. The underlying epithelium is apparently healthy. The basal layer is perfectly regular and there is no hypertrophy or hyperplasia of the epithelial cells. The derma consists of dense collagen in which there are a few collections of lymphocytes and plasma



FIG. 2.—Skiagram showing atrophy of phalanges of little toes and calcification of the first dorsal metatarsal arteries.



FIG. 3.—Cleft in plantar aspect of left little toe. An accumulation of keratin is present between the edges of the epithelium. Granulation tissue, with foreign body giant cells, extends down to the periosteum.  $\times 37$  approx.



FIG. 4.—Left little toe. Chronic periostitis with foreign body giant cells.  $\times 75$ .



FIG. 5.—Left little toe. Dense fibrous tissue enclosing nerve trunks and a narrowed sclerotic artery.  $\times 75$ .

cells. There is no fibroblastic activity. There is very little subcutaneous adipose tissue and dense collagen of the derma merges with that of the periosteal tissue. All the arteries have very thick walls and narrow lumina: in some the thickness is due to great muscular hypertrophy while in others there is subintimal fibrosis. There is no thrombosis. The bone of the phalanx has atrophied very considerably, measuring only  $1 \times 3$  mm. in transverse section. The remaining bone does not appear abnormal. Sections stained with bacterial stains fail to show any bacteria or fungi in the epithelium or in the keratin.

A section through the *site of constriction*, towards the head of the proximal phalanx, shows the same keratinization and a cleft passing right through the epithelium of the plantar aspect. This cleft is lined by granulation tissue and reaches to within a quarter of a millimeter of the periosteum. The granulation tissue has evidently been present for a long time and contains numerous foreign body giant cells. There is a chronic periostitis surrounding the phalanx, the reaction being similar to that in the skin wound: many foreign body giant cells are present. There is no necrotic material other than that in the skin cleft and bacterial stains show no organisms. In this section the subcutaneous collagen is very dense and acellular: it encloses nerves and blood vessels and is firmly attached to the periosteum. The bone here measures 4 mm. in diameter and is rarefied, there being no complete layer of compact bone beneath the periosteum.

A section through the *middle phalanx of the left toe, distal to the constriction*, shows a normal epithelium and no dermal fibrosis. There is a large panniculus adiposus. The blood vessels show the same thickening as that seen in the other sections and here again there is no thrombosis. The bone measures  $6 \times 4$  mm. and its compact outer layer is intact; the cancellous bone is very scanty. There is no periostitis.

*Right Toe.*—Similar sections show the same cutaneous changes as those in the left toe but to a lesser degree. There is excessive keratinization on the plantar aspect at the site of constriction but no breach of the skin surface and only a moderate fibrosis of the derma. There are no definite arterial changes and the bone does not appear rarefied: it measures  $3 \times 5$  mm. at the site of constriction, in the middle of the proximal phalanx. There is no periostitis.

## DISCUSSION.

The patient had evidence of general peripheral vascular disease, with intermittent claudication, but the changes in the little toes seemed to be largely of local origin and there was no suggestion of arteriosclerotic gangrene. The delay in healing after amputation of the toes may however have been due to primary vascular disease.

SPINZIG (loc. cit.) in his discussion of the aetiology of the condition says that some cases have been recorded in which there was an associated skin condition involving other parts of the body. It is possible that any chronic inflammatory condition causing a breach in the skin of the toes may produce fibrous constriction in a negro who is inclined to keloid formation but none of the theories so far advanced explains satisfactorily the almost invariable limitation of the lesion to the little toe.

It occurred to us that this is the localization of the lesions of the form of epidermophytosis commonly known as "athlete's foot" and we hoped that examination of the desquamating epithelium might reveal this fungus. Unfortunately our results were negative but we would like to suggest that such an examination in cases of ainhum may be worth while. A chronic epidermophytosis in a negro might be expected to produce an excess of scar tissue and thus constriction of the toe. In the present case the general peripheral vascular disease:





TRANSACTIONS  
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COMMUNICATIONS.\*

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A SURVEY OF YELLOW FEVER IMMUNITY IN UGANDA.

BY

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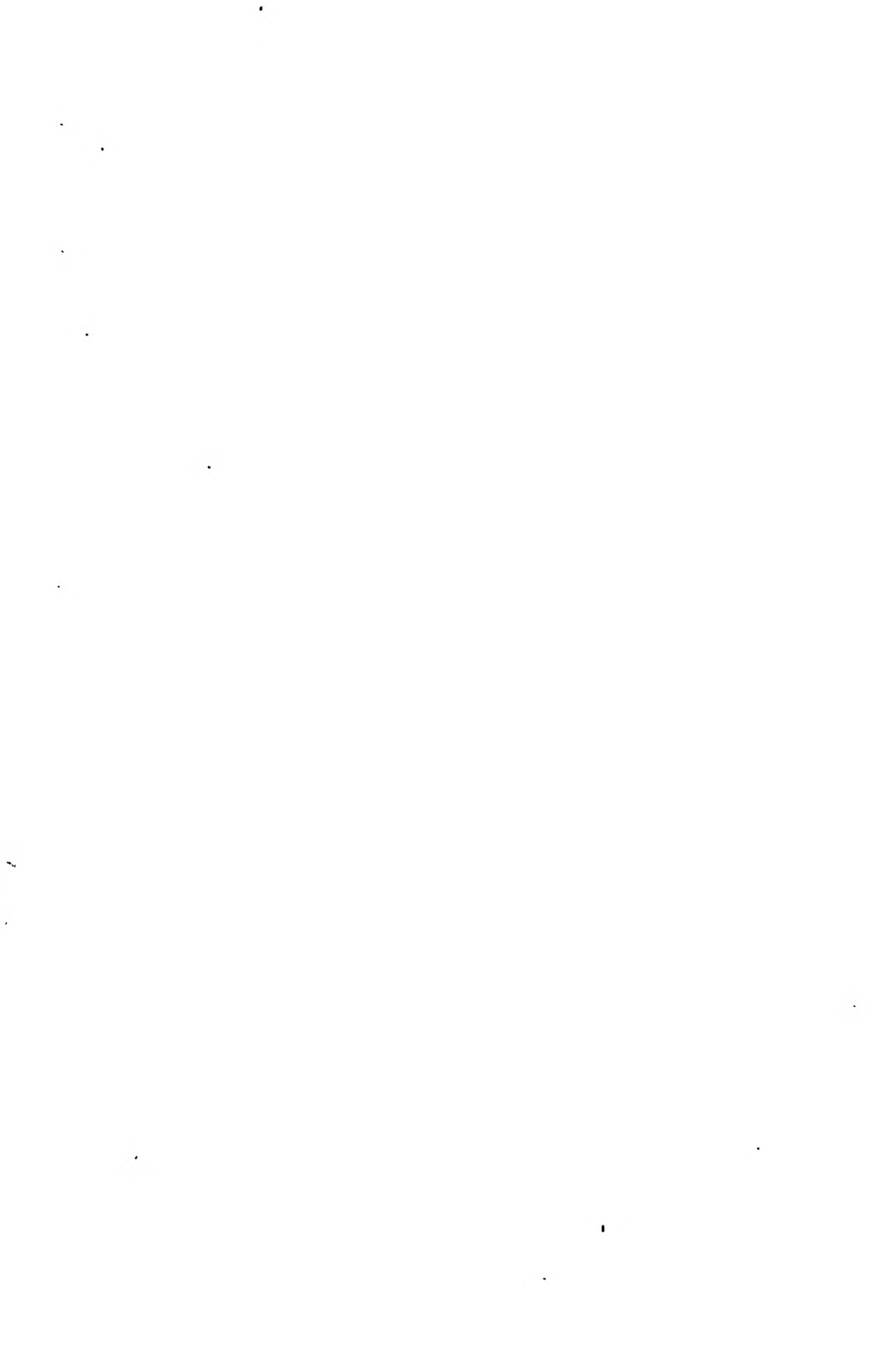
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HISTORICAL.

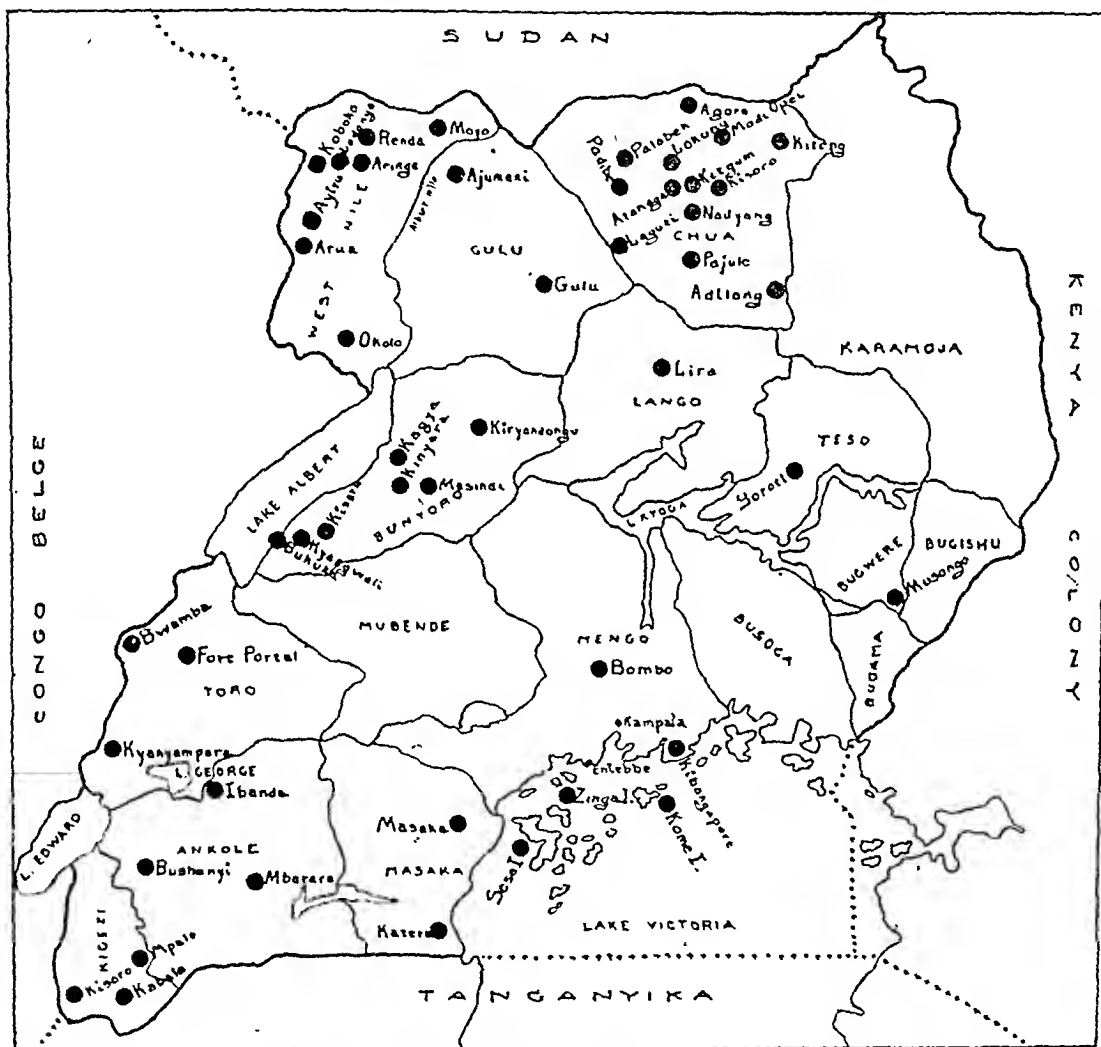
Investigation of the world-wide distribution of yellow fever immunity in man, as an indication of recent infection, was begun by the International Health Division of The Rockefeller Foundation in 1931 with the co-operation of the governments concerned. In 1936 a report was published presenting the results of surveys in North, East, and South Africa (SAWYER and WHITMAN, 1936). Surveys were made also in West Africa (BEEUWEES and MAHAFFY, 1934; and BOYÉ, 1933), the French Cameroons, French Equatorial Africa, the Belgian Congo, and Angola (BEEUWEES, MAHAFFY, BURKE and PAUL, 1934), the Belgian

\* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

† This Institute is supported jointly by the International Health Division of the Rockefeller Foundation and the Medical Department of the Uganda Protectorate.



The eastern, central, and south-western portions of the Protectorate show no evidence of previous or present infection with yellow fever. In the districts of Ankole and Kigezi, in the south-west, there were no immunes among the 297 children and only two among the sixty-nine adults tested. At Katera, in the southern part of Masaka District, there were no immunes among forty-one children and thirteen adults. Eastern Uganda is represented by collections



MAP 1.—Uganda Protectorate.

in Lango, Teso, and Bugwere Districts. In this combined area there were no immunes among seventy-four children and but two among fifty-six adults. Mengo District, in central Uganda, excluding the islands in Lake Victoria, had no immunes among fifty children and but one immune among seventy-four adults.

Congo (MOUCHET, VAN HOOF, DUREN, FORNARA, CLAREBOUT, HENRY, and HENRARD; 1934), and the Anglo-Egyptian Sudan (HEWER, 1934; and FINDLAY, 1938).

In the study of immunity to yellow fever in Africa it was established that there is a zone of prevalence of immunity extending roughly from latitude 6° South to 15° North, and from the Atlantic Ocean eastward to about the 33rd meridian of East longitude. This zone enters western Uganda but does not extend into the main portion of the Protectorate.

In the West African portion of this zone of immunity, cases of yellow fever corresponding to the classical descriptions have been seen frequently and are widely distributed. In the eastern portion of the zone clinical cases of yellow fever had never been observed up to the time of this study, nor had there been recorded any epidemics of an unknown disease which might be presumed to have been yellow fever.

#### ORGANIZATION OF PRESENT STUDY.

To investigate this situation The Rockefeller Foundation, in 1936, at the invitation of the British Colonial Office, established the Yellow Fever Research Institute at Entebbe, Uganda, with close co-operation and financial assistance from the Uganda Government. Included in the programme of this Institute was (1) a more precise delimitation of the zone of yellow fever immunization in the Uganda Protectorate and the neighbouring territories, (2) an inquiry into the type of yellow fever infection giving rise to these immunizations, with particular emphasis on the possibility that it may be mild and differ materially from the classical severe form, (3) determination, if possible, of the factors that limit the spread of this disease through East Africa, and (4) investigation of the degree of danger of spread to the uninfected portions of Africa and to India. The present communication deals with the first of these projects.

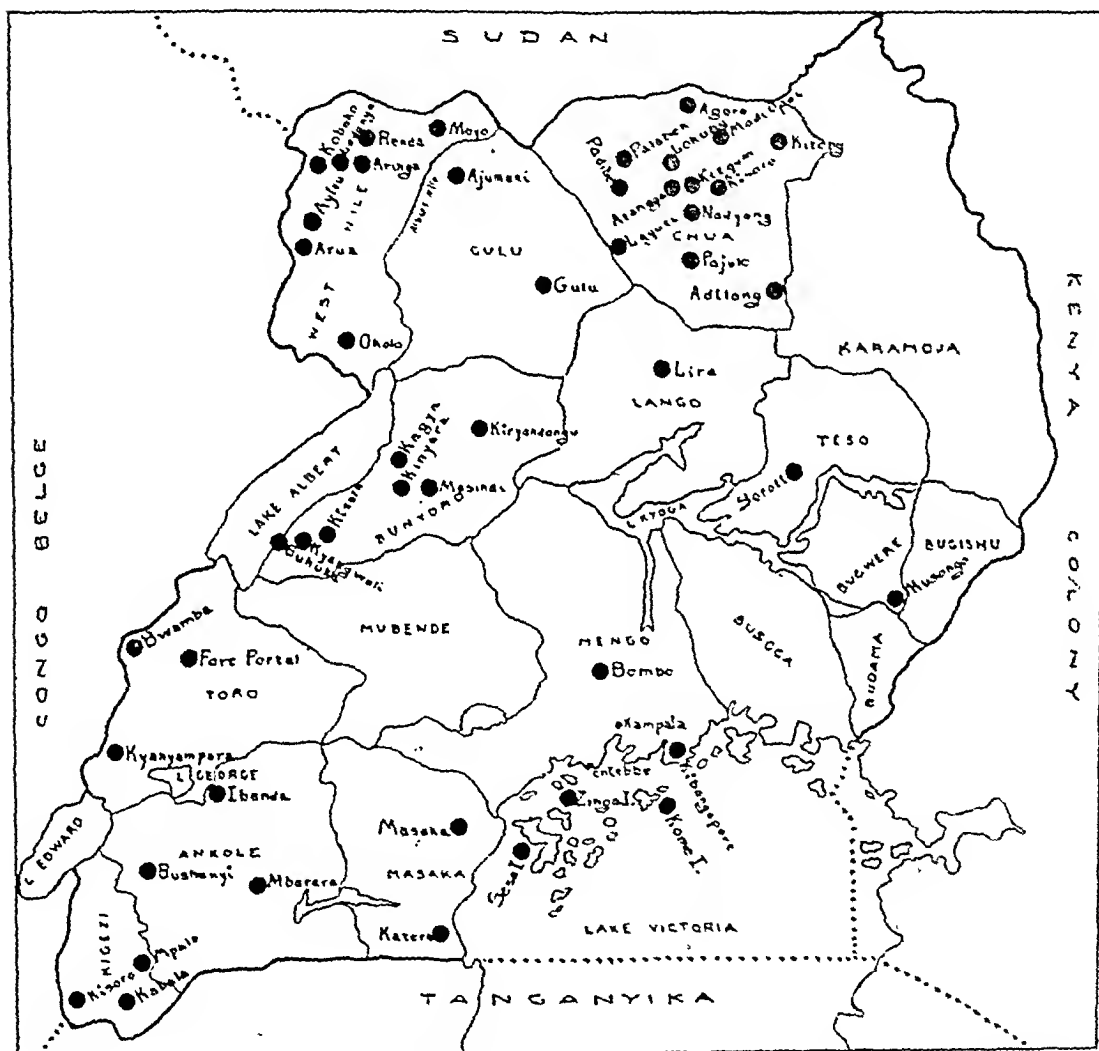
#### *Methods.*

Blood intended to provide serum for a protection test was drawn into vacuum venules of 30 ml. capacity. As opportunity presented, these venules were sent to the Entebbe Laboratory where the serum was separated and the actual tests were done. The method of testing sera was that described by SAWYER and LLOYD (1931) as slightly modified by SAWYER and WHITMAN (1936).

#### RESULTS OF IMMUNITY SURVEY IN UGANDA.

To date, sera from forty-nine different localities in the Uganda Protectorate have been studied, and 3,941 specimens have been tested. These locations are shown in Map 1, and the results are presented in Table I.

The eastern, central, and south-western portions of the Protectorate show no evidence of previous or present infection with yellow fever. In the districts of Ankole and Kigezi, in the south-west, there were no immunes among the 297 children and only two among the sixty-nine adults tested. At Katera, in the southern part of Masaka District, there were no immunes among forty-one children and thirteen adults. Eastern Uganda is represented by collections



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TABLE I

RESULTS OF YELLOW FEVER PROTECTION TESTS ON SERA FROM RESIDENTS OF UGANDA

Town.	Location (Map reference).	Age group.*	Number tested.	Number immune.	Per cent. immune.	Age in years of youngest donor of immune serum.
<i>West Nile District.</i>						
Koboko ...	1	Adults	74	1	1.3	16
Lodonga Mission	2	Children	25	0	0.0	
Aringa ...	3	Children	20	0	0.0	
		Adults	56	3	5.3	24
Renda ...	4	Children	9	0	0.0	
		Adults	16	0	0.0	
Moyo ...	5	Children	19	0	0.0	
		Adults	32	1	3.1	30
Ayivu ...	7	Children	68	0	0.0	
Arua ...	8	Children	25	0	0.0	
		Adults	67	4	6.0	25
Okolo ...	9	Children	25	0	0.0	
		Adults	24	0	0.0	
<i>Chua District.</i>						
Agoro ...	11	Children	25	0	0.0	
		Adults	22	3	13.6	28
Madi Opei ...	12	Children	23	0	0.0	
		Adults	25	0	0.0	
Kiteng ...	13	Children	25	0	0.0	
		Adults	25	0	0.0	
Palabek ...	14	Children	25	0	0.0	
Lokung ...	15	Children	25	0	0.0	
		Adults	25	1	4.0	22
Padibe ...	16	Children	25	0	0.0	
		Adults	25	0	0.0	
Atanga ...	17	Children	12	0	0.0	
Kitgum ...	18	Children	20	0	0.0	
		Adults	30	3	10.0	25
Kisoro Mission...	19	Children	17	0	0.0	
Nadyang ...	20	Children	5	0	0.0	
Laguti ...	21	Children	6	0	0.0	
Pajule ...	22	Children	30	2	6.7	10
Adilang ...	23	Children	25	0	0.0	
<i>Gulu District.</i>						
Ajumani ...	6	Children	17	0	0.0	
		Adults	29	0	0.0	
Gulu ...	10	Children	21	0	0.0	
		Adults	30	0	0.0	

\* "Children" include persons 14 years of age or younger.

"Adults" include all persons over 14 years of age.

TABLE I—(continued).

Town.	Location (Map reference).	Age group.*	Number tested.	Number immune.	Per cent. immune.	Age in years of youngest donor of immune serum.
<i>Lango District.</i>						
Lira ... ..	24	Children	24	0	0·0	25
		Adults	30	1	3·3	
<i>Teso District.</i>						
Soroti ... ..	32	Children	25	0	0·0	
<i>Bugwere District.</i>						
Musongo ... ..	33	Children	25	0	0·0	30
		Adults	26	1	3·8	
<i>Bunyoro District.</i>						
Kiryandongo ...	25	Children	92	2	2·2	9
		Adults	41	2	4·9	
Kagya ... ..	26	Children	11	1	9·1	13
		Adults	24	0	0·0	
Kinyara ... ..	27	Children	24	0	0·0	10
		Adults	17	0	0·0	
Masindi... ..	28	Children	25	1	4·0	
		Adults	25	0	0·0	
Buhuka ... ..	29	Adults	19	1	5·3	" Adult "
Kyangwali ... ..	30	Children	40	0	0·0	
Kisaru ... ..	31	Children	44	0	0·0	
<i>Toro District.</i>						
Bwamba County	35	Children	961	19	2·0	6
		Adults	472	90	19·0	
Fort Portal ...	36	Adults	21	2	9·5	30
Kyanyampara ...	37	Not given	49	1	2·0	?
<i>Mengo District</i>						
Bombo ... ..	34	Adults	74	1	1·4	35
Kibangaport ...	49	Children	50	0	0·0	
Kome Island ...	48	Children	53	0	0·0	
		Adults	42	5	11·9	?
Busi and Zinga Islands ... ..	47	Adults	9	0	0·0	
<i>Masaka Districts.</i>						
Katera ... ..	44	Children	41	0	0·0	
		Adults	13	0	0·0	
Masaka ... ..	45	Children	35	0	0·0	

\* " Children " include persons 14 years of age or younger.

" Adults " include all persons over 14 years of age.



TABLE I—(continued).

Town.	Location (Map reference).	Age group.*	Number tested.	Number immune.	Per cent. immune.	Age in years of youngest donor of immune serum.
Sese Islands ...	46	Adults	225	5	2.2	25
		Adults	76	1	1.3	22
<i>Ankole District.</i>						
Ibanda ...	38	Children	46	0	0.0	
		Adults	14	0	0.0	
Bushenyi ...	39	Children	41	0	0.0	
		Adults	10	0	0.0	
Mbarara ...	40	Children	56	0	0.0	
		Adults	7	0	0.0	
<i>Kigezi District.</i>						
Mpalo ...	41	Children	52	0	0.0	
		Adults	5	0	0.0	
Kisoro ...	42	Children	31	0	0.0	
		Adults	4	0	0.0	
Kabale ...	43	Children	71	0	0.0	
		Adults	29	2	6.9	?

\* "Children" include persons 14 years of age or younger.

"Adults" include all persons over 14 years of age.

The islands in Lake Victoria present a more complex situation. During the sleeping sickness epidemic of 1906 these islands were completely depopulated and the inhabitants were transferred elsewhere in Uganda. After this disease was brought under control, people were again allowed to occupy the islands. However, most of the new settlers had not lived there previously but came from other parts of Uganda. Resettlement continued over a long period of time. Consequently, such of the older people as show yellow fever immunity may have acquired it elsewhere. Among fifty-three children from Kome, Busi, Zinga, and the Sese Islands none were immune; among 127 adults six were immune.

The town of Masaka, in Masaka District, has been of particular interest, since there is some suspicion that cases of yellow fever occurred there in 1936. However, tests for immunity on sera from thirty-five Masaka children gave negative results. Five out of 225 adults were immune, giving an immunization rate of 2.2 per cent.

The portions of Uganda bordering the Sudan show a somewhat higher percentage of immunes. In Chua District, through which passes the main highway from the Sudan, there were two immunes among 263 children. The age of the younger immune was estimated at 10 years. From 152 adults there were 7 protective sera, or 4.6 per cent. Sera from thirty-eight children and

fifty-nine adults in Gulu District were all non-immune. In the West Nile District there were no immunes among 181 children ; but among adults nine out of 269 were immune, or 3·2 per cent.

In western Uganda sera were collected from Bunyoro and Toro Districts. For our consideration, Toro District may be divided into two parts : (1) the main portion lying east of the Ruwenzori Range, and (2) Bwamba County, which lies west of the mountains and is, essentially, an extension of the Congo Basin into Uganda. In the second area our most extensive investigations have been made, and the results obtained will be presented in detail in a subsequent section of this communication. The main portion of Toro District is represented by 70 sera, of which 3 were protective. In Bunyoro District somewhat different results were obtained. Among sera from 236 children, there were four immunes. The age of the youngest donor of an immune serum was estimated at 9 years. This indicates an immunization rate, among children, of 1·7 per cent. Among sera from adults there were three immunes among 126 specimens, or 2·4 per cent. In view of the large number of sera tested it is apparent that there has been no extensive immunization in recent years, if at all.

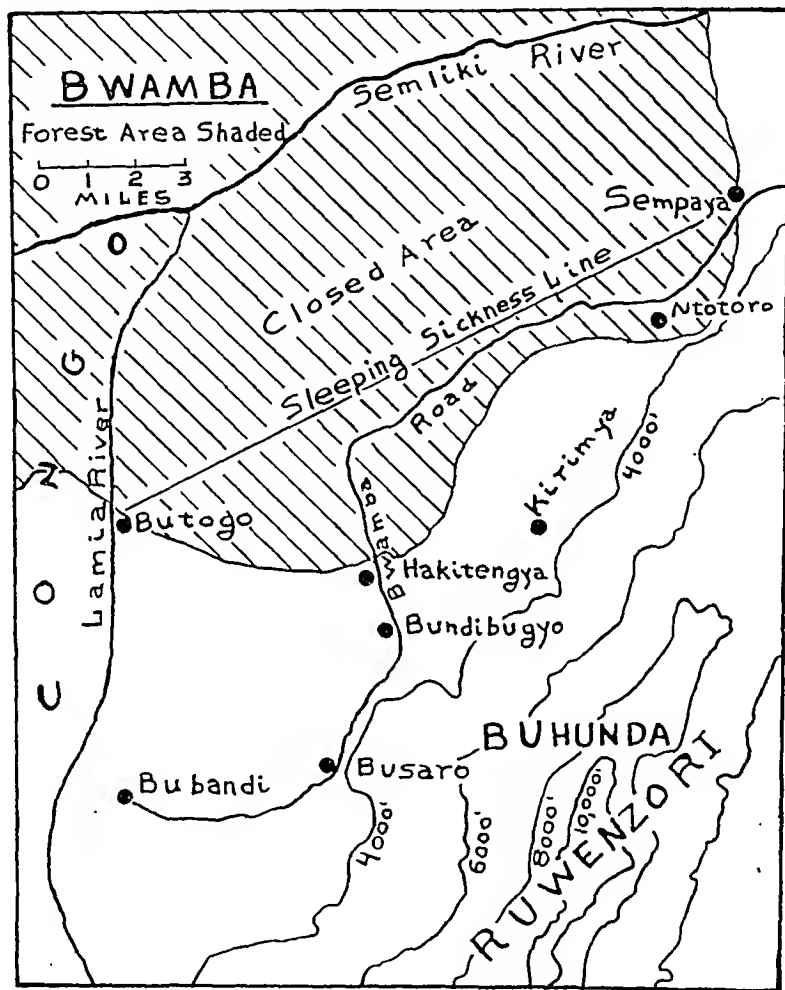
It must be borne in mind that the Uganda Protectorate occupies only a small area. The greatest distance from east to west is about 275 miles and from north to south about 350 miles. There is much travel among natives, and consequently many of the residents of one district have spent some time in other parts of Uganda or in the neighbouring territories.

From the results presented, it appears that the main portion of Uganda, comprising the eastern, central, and south-western sections, shows no evidence of past or present yellow fever infection. In northern Uganda there is an appreciable degree of immunization among children and adults. These immunizations may be a reflection of travel into the infected areas or, to some extent, of isolated infections resulting from an extension of the zone of immunization southward from the Sudan. In western Uganda there may be some evidence of yellow fever infection at some previous time but none of present infection.

#### STUDIES IN BWAMBA COUNTY, TORO DISTRICT.

The Bwamba forest area of Toro District is shown in Map 2. Geographically, Bwamba represents a continuation of the Ituri Forest of the Belgian Congo into Uganda. The Congo-Uganda boundary consists of two small rivers, the Semliki and the Lamia, both shallow enough in parts to permit easy passage. The remaining Bwamba boundary, which separates Bwamba from the rest of Toro District, is the 4,000 foot contour of the Ruwenzori Range. Approximately two-thirds of the 75 square miles of Bwamba is forested. The remaining portion consists of grassy plains, cut by wooded ravines and becoming more undulating as the base of the mountain is approached. About two-thirds of the Bwamba forest is a " Sleeping Sickness Area " in which no one is permitted to live.

The total population of Bwamba County is estimated to be 25,000. There are no real towns, but the inhabitants are grouped, for the most part, around centres such as Hakitengya, Busaru, and Buhandi. These centres are all outside of the forest area and toward the base of Ruwenzori. The forests are said to be inhabited by nomadic pygmies who are not completely under government



MAP 2.—Bwamba Forest Area, Toro District.

supervision. There are, however, groups of people living on the edge of the forest, mainly between Butogo and Hakitengya. The population of Bwamba consists mainly of members of the Baamba tribe, who also occupy the adjoining portion of the Congo. Consequently, there is constant passage to and from the Congo to visit relatives and friends.

Bwamba Country is divided into four gombololas, with headquarters at

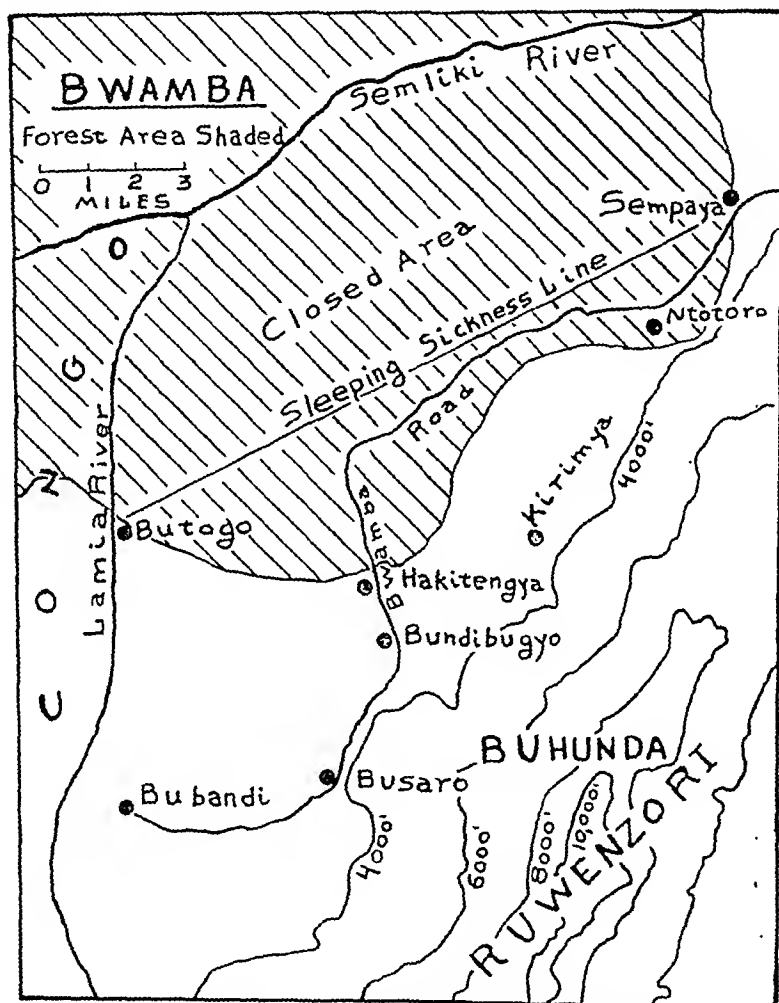
Hakitengya, Bubandi, Busaro, and Kirimya. Although technically not in Bwamba County, many of the Bakonjo tribe, living on the slopes of Ruwenzori in Buhundu County, patronize the market at Bundibugyo and make use of the Government Dispensary there. Their own headquarters are situated on the eastern slope of the mountain and, rather than cross over the mountain, they make use of the facilities of Bwamba.

We have tested 1,231 sera from residents of Bwamba on whom we have information regarding residence and age. We have excluded from this summary the results of tests on 59 sera from donors on whom information was lacking. One hundred and nine of these 1,231 sera were protective. On this basis, combining all age groups and places of residence, 8.9 per cent. of this population is immunized against yellow fever. The data, grouped by donor's age and residence, are presented in Table II.

TABLE II  
RESULTS OF YELLOW FEVER PROTECTION TESTS WITH SERA FROM BWAMBA COUNTY

Area.	Age group.	Sera tested	Number protective	Per cent. protective.
Hakitengya ... ..	0- 4	2	0	0.0
	5- 9	21	3	14.3
	10-14	14	1	7.1
	15 and over	91	23	25.3
Busaro and Butogo ...	0- 4	22	0	0.0
	5- 9	157	4	2.5
	10-14	173	5	2.9
	15 and over	202	40	19.8
Bubandi ... ..	0- 4	3	0	0.0
	5- 9	66	2	3.0
	10-14	166	3	1.8
	15 and over	78	21	26.9
Kirimya ... ..	0- 4	6	0	0.0
	5- 9	36	0	0.0
	10-14	62	1	1.6
	15 and over	50	4	8.0
Buhundu ... ..	0- 4	1	0	0.0
	5- 9	14	0	0.0
	10-14	16	0	0.0
	15 and over	51	2	3.9
Total for all Bwamba...	0- 4	34	0	0.0
	5- 9	294	9	3.1
	10-14	431	10	2.3
	15 and over	472	90	19.0
		1,231	109	8.9

The total population of Bwamba County is estimated to be 25,000. There are no real towns, but the inhabitants are grouped, for the most part, around centres such as Hakitengya, Busaru, and Buhandi. These centres are all outside of the forest area and toward the base of Ruwenzori. The forests are said to be inhabited by nomadic pygmies who are not completely under government



MAP 2.—Bwamba Forest Area, Toro District.

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	15 and over	472	90	19.0
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It is apparent that the distribution of yellow fever immunity among the five areas is unequal. In Kiriya Gombolola there was but one child out of 104 immunized, while in Buhundu there were none among the thirty-one tested. The rate of immunization for adults in these two areas is also low, representing 6 per cent. of the combined totals. These two areas are out of contact with the forest. All of the remaining areas contain portions of the forest, and the great majority of the protective sera collected in these areas came from settlements on the forest border such as Hakitengya and Butogo. Out of 371 sera from adults of this area eighty-four were protective, giving an immunization rate of 22.6 per cent. Among 624 children tested the immunization rate was 2.9 per cent. The age of the youngest immune was 6, but only a few children younger than 6 were tested. The connection, in Bwamba, between the forest and yellow fever immunization appears to be quite definite.

### DISCUSSION.

It is immediately obvious that there is a marked difference in the degree of immunization between adult and child groups. This difference holds in all areas where there is extensive immunization. While there is no significant change in the immunization rate between the 4 to 9 year and the 10 to 14 year groups, the immunization rate for adults is almost ten times that for children. This fact may have either of two explanations: (1) There is some change in habit or occupation at maturity which increases the risk of yellow fever infection, similar to the circumstances occurring in "jungle type" yellow fever infections in South America (SOPER, 1936), or (2) there was an extensive wave of infection over 15 years ago resulting in a persistent residual infection, quite probably with an animal reservoir and with only occasional transfer to human beings. Either of these two alternatives would imply an intimate association with the forest.

In this connection it is of interest to note that two mosquitoes known to be efficient transmitters of yellow fever, *Aedes simpsoni* (PHILIP, 1929) and *Eretmopodites chrysogaster* (BAUER, 1928), occur in relatively large numbers in the Bwamba Forest. These two species combined represented 12.2 per cent. of a sample of 252 mosquitoes caught there with human bait. In banana plantations near dwellings 97.2 per cent. of the mosquitoes caught were *Aedes simpsoni*. *Aedes aegypti*, although present, has been observed but rarely. These observations on the distribution of mosquitoes in Bwamba were made by Mr. J. O. HARPER, of the Kenya Medical Service. Although we have as yet tested sera from only five wild monkeys caught in Bwamba (*Cercopithecus ascanius schmidtii*), the serum of one of them was protective against yellow fever virus.

Extensive attempts, as yet unsuccessful, have been made to isolate the virus causing these immunizations in Bwamba. During 1937, while the Bwamba road was under construction between Sempaya and Hakitengya, one of us

(A.F.M.) kept the road labourers under medical observation for a period of 6 months. During this time many cases of pyrexia of an unknown origin were observed, and samples of serum were taken from over 100 of these cases and injected into mice. Although the sera yielded no yellow fever virus, a new viral disease described as "Bwamba fever" was encountered, and its etiological agent has been investigated at the Entebbe Laboratory (SMITHBURN, MAHAFFY, and PAUL, 1940). During 1938 and part of 1939 all patients visiting the Bundi-bugyo Dispensary who were found to have fever without any obvious cause were bled and their serum was injected into mice. A total of 724 such injections was made with negative results. As a check to determine whether yellow fever infections were being seen but not demonstrated by the mouse injection method, we attempted to secure second ("convalescent") bleedings from as many patients as possible who had non-protective sera at the time of the illness for which they sought dispensary treatment. We secured seventy-eight such second specimens, and all were still non-protective. The interval between the first and second bleedings ranged from 21 to 161 days, and the average time was 100 days.

As a further check we have obtained specimens of liver tissue for pathological examination from 150 residents of Bwamba who had died after short illnesses. In no case were pathological lesions observed which were characteristic of yellow fever infection.

#### SUMMARY.

From the data presented it appears that eastern, central, and south-west Uganda have been free from yellow fever infection. In the northern and western portions of the Protectorate there is a low incidence of immunity which does not reflect present infection with this virus. These regions adjoin areas outside Uganda where the incidence of yellow fever immunity is known to be high. In only one part of Uganda, that portion of Toro District west of the Ruwenzori Range and bordering the Congo Forest, is there any cause to suspect a continuing yellow fever infection, possibly endemic in character. Even in this area extensive investigation over a period of 3 years has failed to reveal an active case of yellow fever infection.

#### *Acknowledgement.*

Throughout this survey we were fortunate to have had the active support and generous co-operation of the Director of Medical Services of Uganda, Dr. W. H. KAUNTZE, and his administrative staff, as well as various District Medical Officers of the Protectorate, to many of whom we are indebted for aid in the collection of sera. Most of the sera tested were collected by Drs. A. F. MAHAFFY, A. W. BURKE, H. R. JACOBS, K. C. SMITHBURN, and T. P. HUGHES, and Mr. E. G. GIBBINS. Protection tests were participated in by all



of the above, and, in addition, by Dr. J. H. PAUL. Some data relative to Uganda contained in SAWYER and WHITMAN's survey have been included in the map of Uganda and in Table I.

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## THE FIRST RECOGNIZED EPIDEMIC OF YELLOW FEVER.

BY

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The first recognized account of yellow fever is that given by Père JEAN BAPTISTE DU TERTRE (1667-1671) of the outbreak which he himself witnessed in the Island of Guadeloupe in 1648. A few months later, DIEGO LÓPEZ DE COGULLUDO (1688) described an epidemic with similar characteristics in Yucatan. Since CARTER (1931) published his epidemiological and historical study of the place of origin of yellow fever, much has been learnt not only of the nature of the disease but of the circumstances attending the outbreak of yellow fever in 1648. These observations are in favour of the view that infection was brought to the West Indies from the West Coast of Africa, either to Barbados or less probably to St. Christopher, whence it spread to Yucatan and to Havana. Before discussing the evidence on which this conjecture is based, it is necessary to retail briefly the accounts, now well known, of the outbreaks in Guadeloupe and Yucatan.

### THE OUTBREAK IN GUADELOUPE.

The islands of Guadeloupe and Martinique had been colonized by the French in June and July, 1635, respectively. Food for only 2 months was brought by the original colonists from Dieppe and, as a result, conditions soon became difficult, more especially as the natives with whom the colonists quarrelled fled and refused to supply food. DU TERTRE, who arrived in Guadeloupe in 1640, wrote two works on the history of the Caribbean: (I) "Histoire generale des isles des Christophe, de la Guadeloupe, de la Martinique et autres dans l'Amerique, 1654," and (II) "Histoire generale des Antilles habitées par les François, 1667-1671." The early history of Guadeloupe is described in both books. The famine which began 2 months after the arrival of the colonists caused them, for lack of potatoes or manioc, to live on grass and fresh tortoise meat. This diet, probably as a result of vitamin deficiency, caused acute dysentery and death ("flux de ventre et de sang qui en firent mourir plusieurs"). The famine went on for five years, till 1640, and was followed by an almost general mortality to which, beside the famine, two things especially contributed. The first was a certain disease commonly called in the islands the coup de barre, characterized by a very severe headache, associated with violent pulsations in the arteries of the temples and great difficulty in breathing, with lassitude and pain in the thighs as if one had been

struck with blows by a bar. The disease attacked especially those who were digging. The cruelty of the overseers was the other cause of death, not only of "ces pauvres engagez," but of the gentlemen of the Company and the merchants of Dieppe, for the colonists were forced by blows and by severity to work in clearing the woods and in all weathers. The French, in fact, were treated worse than slaves in Barbary. The colony at Martinique had no famine and no disease.

Although the term "coup de barre" was often applied later to one of the symptoms of yellow fever, the malady described by DU TERTRE as present in Guadeloupe in 1640 suggests either dengue or more probably beriberi resulting from food deficiency. BRETON (1665) also noted and described the "coup de barre" of Guadeloupe in his Carib-French dictionary, but since his work was published after DU TERTRE's first book, it is not quite correct, as CARTER (1931) suggests, to assert that the latter borrows entirely from BRETON. No other event of medical importance in Guadeloupe is mentioned by DU TERTRE till 1648, "During this same year, 1648, the plague (la peste), hitherto unknown in the islands since they were inhabited by the French, was brought thither by certain ships." It began in St. Christopher, and in the 18 months that it lasted there it carried off nearly one-third of the inhabitants. A ship, "Le Boeuf," of Rochelle, carried it from St. Christopher to Guadeloupe, the sailors and passengers being sick and dying on board her. When the boat arrived at Basseterre, Père ARMANDE DE LA PAIX, the Superior of the Jesuit Mission, went aboard "to confess and serve the sick and dying." The Father contracted the disease and died on St. Dominic's Day, 4th August. The disease was contagious in Guadeloupe and lasted 20 months. The symptoms were violent pain in the head, general weakness in all the limbs, "and continual vomiting so that in three days it would send a man to his grave." No actual mention is made of "black vomit."

#### THE OUTBREAK IN BARBADOS.

There is thus clear evidence that an infectious disease was carried from St. Christopher to Guadeloupe in July, 1648. Unfortunately, there is no clear description of the disease in St. Christopher apart from the fact that it was similar to the epidemic occurring in Barbados.

The two chief accounts of the Barbados outbreak are given by RICHARD LIGON (1657), who was an actual observer, and by JOHN SCOTT (1634-1696), whose account was derived from eye-witnesses. LIGON left São Thomé on 10th August with slaves for Bridgetown. On arrival, the town seemed very prosperous. "Yet notwithstanding all this appearance of trade, the Inhabitants of the Islands and shipping too (there were twenty-two good ships in harbour) were so grievously visited with the plague (or as killing a disease) that before a month was expired after our Arivall, the living were hardly able to bury the dead. Whether it were brought thither in shipping: (for in long voyages,

diseases grow at sea and take away many passengers, and those diseases prove contagious), or by the distempers of the people of the Iland : who by the ill dyet they keep and drinking strong waters, bring diseases upon themselves, was not certainly known. But I have this reason to believe the latter ; because for one woman that dyed there were ten men and the men were the greater deboystes. In this sad time we arriv'd in this Iland ; and it was a doubt whether this disease or famine threatned most ; there being a generall scarcity of victuals throughout the whole Iland." LIGON had proposed to go on to Antigua, " where we intended to plant : but the ships being (for the most part) infected with this disease we were compelled to stay longer." At the time of their arrival at Bridgetown and a month or two after " the sickness raign'd so extremely that the dead carcasses were thrown into the bog, whereby the water was infected." SCOTT (1667) adds but few details : he, too, noted that men were more frequently attacked than women, " as is usual with epidemics, showing a favour to that sex."

Although LIGON and SCOTT constitute the two most important authorities for the outbreak in Barbados, there are numerous other contemporary references to the epidemic which is shown to affect both Barbados and St. Christopher. JOHN WINTHROP (1853), the Governor of Massachusetts, left manuscript notes for a history of New England from 1630 to 1649. Incidentally, WINTHROP was a man of scientific interests and was the first to import chemical apparatus into North America (CHILD, 1940). Under the year 1647 he wrote, " An epidemical sickness was through the country (New England) among Indians and English, French and Dutch. It took them like a cold and a light fever with it. Such as bled or used cooling drinks died ; those who took comfortable things, for the most part recovered and that in few days. Wherein a special providence of God appeared, for not a family nor but few persons escaping it, had it brought all so weak as it did some, and continued so long, our hay and corn had been lost for want of help ; but such was the mercy of God to His people, as few died, not above forty or fifty in Massachusetts and near as many at Connecticut." Mrs. Winthrop, aged 56, however, was one of those who died. This outbreak, which occurred in the summer, was probably influenza and was quite distinct from the Caribbean epidemic. This point must be insisted upon since HUTCHINSON (1764), WEBSTER (1804) and others completely confuse the two.

Later WINTHROP continues, referring to the winter months. " It pleased the Lord to open to us a trade with Barbados and other Islands in the West Indies, which as it proved gainful, so the commodities we had in exchange there for our cattle and provisions as sugar, cotton, tobacco and indigo were a good help to discharge our engagements in England. And this summer there was so great a drouth as their potatoes and corn, etc., were burnt up ; and divers London ships which rode there were so short of provisions as, if our vessels had not supplied them, they could not have returned home. . . . After the

great dearth of victuals in these islands followed presently a great mortality (whether it were the plague or pestilent fever, it killed in three days) that in Barbados there died six thousand and in Christophers of French and English, near as many and in other islands proportionable. The report of this coming to us, by a vessel which came from Fayal, the court published an order that all vessels which should come from the West Indies should stay at the castle and not come on shore, nor put any goods on shore, without license of three of the council, on pain of one hundred pounds, nor any to go aboard, etc., except they continued there, etc., on like penalty. The like order was sent to Salem and other haven towns." The order is of considerable interest since it represents the first instance in which quarantine regulations were applied in the New World. The reasons for promulgating the order (cf. Records of the Governor and Company of the Massachusetts Bay, 1853) were stated to be "For as much as this Court is credibly informed yt ye plague or like greivous infectious disease, hath lately exceedgly raged in ye Barbadoes, Christophers and other islands in ye West Indies, to ye great depopulatg of those, it is therefore ordred, yt all our own or othr vessels comeing from any pts of ye West Indies to Boston harbor shall stop and come to an anchor before they come at ye Castle . . . ." It was nearly two years, that is to say in the autumn of 1649, before the order was repealed. "The Court doth think meete that the order concerning the stoping of West Indie ships at the Castle should hereby be repealed, seeing it hath pleased God to stay the sickness there."

Two letters throw further light on the outbreak. Mr. RICHARD VINES, who had lately removed from New England to Barbados, wrote to Governor WINTHROP, giving him some account of the epidemic. His letter, dated "Barbadoes, April 20, 1648," states "The sickness was an absolute plague, very infectious and destroying insomuch that in our parish there was buried twenty in a week and many weeks together fifteen or sixteen. It first seized on the ablest men, both for account and ability of body. Our New England men here had their share and so had all nations, especially Dutchmen, of whom died a great company, even the wisest of them. The contagion is well nigh over." That this last statement was not correct is shown by a further letter dated "Salem, 17th December, 1648," from LUCY DOWNING, of Boston, to JOHN WINTHROP, Jr., "My 2 sonns Jo and Robin I bless God are safe returned but Robin in respect of the loss of his master, and Jo in respect to the sad Sickness still at Barbados are both now gone to Boston to see which waye Providence will dispose for them." It is thus clear that an epidemic disease began in Barbados in September, 1647: it was fatal in three days. A similar disease occurred in St. Christopher and thence spread to Guadeloupe in August, 1648. Here also it killed in three days. The epidemic was still present in Barbados in December, 1648, and apparently had not disappeared till the early autumn of 1649 when the quarantine regulations in New England were at last relaxed.

## THE OUTBREAK IN YUCATAN.

The outbreaks in Barbados, St. Christopher and Guadeloupe did not, however, represent the full extent of the epidemic, for a very similar disease made its appearance both in Yucatan and in Havana. The epidemic in Yucatan was very fully reported by LÓPEZ DE COGULLUDO (1668): his account has been translated by FINLAY (1912) and by CARTER (1931). The epidemic, the "peste," began in the beginning of June, 1648, in the City of Campeche, on the west coast of the peninsula of Yucatan. In a few days "the disease" so pressed on it that it was totally laid waste. A letter is quoted from a citizen of Campeche, "If God does not pity our misery and soon soften the rigor of His Justice it will be said 'Here was Campeche,' as it was said of Troy. . . . The roads from Campeche were guarded, fearing communication of the contagion . . . With this fear . . . passed the month of July, in the end of which began some people" (in Merida) "to sicken, who died very soon, but it was not recognized to be the 'peste' until the beginning of August. With such quickness and violence it came on great and little, rich and poor, that in less than eight days almost the whole city was sick at one time and many of the citizens of highest name and authority in it died. The city, afflicted with such a misfortune not seen before since this land was conquered by the Spanish nation . . . sought leave to bring in the Holy Image of Our Lady of Izamal. . . . The most part of the Indians of Izamal who attended the Holy Image on the road and in the City of Merida, were attacked by the contagion of the peste in it and a few days after they arrived at Izamal passed from this present life." MOLINA SOLIS (1904-1910) confirms this by stating that "in September all the district of Izamal was infected. The tribulation of the city (Merida) was very great as never had it experienced such a disaster. . . . In the beginning few of the Friars died . . . when the peste was at its height few were sick . . . afterwards many sickened at once. . . . Pestilences are accustomed to be a common accident in other lands, which uniformly attack all, but it was not thus in Yucatan, which was the occasion of the greater confusion. It is not possible to say what was this malady, because the physicians did not recognize it." Of the symptoms "The most common was for the patients to be taken with a very severe and intense pain in the head and of all the bones of the body, so violent that it appeared to dislocate them or to squeeze them as in a press. In a little while after the pain a most vehement fever, which to most occasioned delirium, although to some not. Followed some vomitings as of putrefied blood and of these very few remained alive. To others there was a flow from the bowels of a bilious humour which "being "corrupted caused dysentery which they call 'sin vómitos'. Others were provoked to them "(vomitings) "with great violence but in vain and many suffered the calentura and pain in the bones without other accidents. . . . To the most the fever appeared to remit entirely on the third day; and they said that already they felt no pain; the delirium ceased, conversing sensibly, but they were not able to eat nor to drink anything and thus going on one or more days, speaking and saying

that they were well, they died. There were many who did not pass the third day; the most died beginning the fifth, very few reached the seventh, except those who survived, and of these the most were elderly. It attacked young men, the most robust and healthy with most violence and finished their lives the quickest. . . . Although very many women sickened, the sickness did not bear as hard on them as on men . . . but it was rare that one found a pregnant woman who remained alive. . . . The sickness killed the most robust youths the quickest. . . . The same year of 1648 in which the peste began some pestilential air or other bad influence had dried up all the pines well-grown and large . . . all the new little pines remaining alive, and then I made this reflection that of the children of tender age whom the peste attacked in Yucatan there were very few who died compared with people of more advanced age." At first the only Indians affected were those in close contact with the Spaniards and those who went into the city. Later "in many of their pueblos the same sickness showed as among the Spaniards, making fearful ravages as among a people without resources or medicines. The sickness lasted in the whole land for the space of two years": almost everyone who came into the country during this time sickened but there were no recurrences. "All remained so pallid that they appeared dead: many without hair, with eyelashes dropped out, all so broken that although they had had only two days of fever and a little pain in the bones . . . many could not regain their strength."

The above description is very characteristic of yellow fever except for the failure to mention jaundice. Many similarities will be noted with the diseases occurring in Barbados and Guadeloupe.

#### THE OUTBREAK IN HAVANA.

The last recorded outbreak at this time was in Havana. Unfortunately, the only authority for the disease in Havana is PEZUELA, who writing nearly 220 years later, is uncertain as to the date of the outbreak he describes. PEZUELA's accounts are in all probability based on the records of VILLALBA the Governor, who, himself, suffered from the disease. In his "Diccionario geográfico, estadístico, histórico de la Isla de Cuba" (Madrid 1863-1866, tom. 3, p. 23), PEZUELA wrote under the year 1648, "Peste of putrid fevers in Havana and in the fleet of Don Juan Pujadas, stationed in the port almost all the summer. Three assessors of government died, namely successively, an alcalde, many functionaries, a third part of the garrison and of civilians in the neighbourhood and an even greater proportional number of the crews and passengers of the vessels." There is nothing chronicled for the year 1649. On the other hand, in the "Historia de la Isla de Cuba," PEZUELA (1868-1878, tom. 2, pp. 106-109) gives no entry for the year 1648 but "in the spring of 1649, supervened to terrify it" (the country) "a horrible epidemic. From that of small-pox which decimated the new born pueblos . . . at the beginning of the sixteenth century,

there had not been known other contagions and sicknesses than those inherent to its warm climate and the malignant fevers of the summer of 1620. The records of Governor Villalba neither detailed nor even explained the symptoms of the sickness which then prevailed in many coastwise settlements of the Continent and which was supposedly introduced into Havana by vessels from Cartagena and Portobello. . . . A third part of its population was devoured from May to October by a species of putrid fever which carried off those attacked in three days. The therapy, tried gropingly by some experimenting physicians . . . against an unknown sickness, aggravated instead of curing it." (CARTER's translation.) VILLALBA, the Governor, fell sick in August, but recovered. The auditor, MOLINA, who was appointed as his temporary successor, died, however, as also did the three licentiates, who successively took his place. As in the previous account, an *alcáde* and many functionaries, a third part of the garrison and of civilians in the neighbourhood and an even greater number of the crews and passengers of the squadron succumbed. The losses in the garrison were replaced by officers and men from Vera Cruz and Cadiz in 1650 : a fact which suggests that 1649 was the more correct date. In addition, the *Historia* (tom. 2, p. 112) states that the fever in Santiago de Cuba in 1653 was three years after the Havana outbreak, while (p. 107) it is also stated that "this peste of putrid fevers had afflicted Vera Cruz and other pueblos of New Spain the preceding summer," *i.e.*, in 1648, when Campeche was infected.

#### THE ORIGIN OF THE PANDEMIC.

The five outbreaks which have been described can obviously be divided into two groups, one involving Barbados, St. Christopher and Guadeloupe in the east, the other Yucatan and Cuba in the west. The descriptions of the outbreaks in Guadeloupe and Yucatan leave no doubt that they were yellow fever. According to DU TERTRE, infection was brought to Guadeloupe from St. Christopher: the outbreak in St. Christopher must therefore almost certainly have been due to yellow fever. The epidemics in St. Christopher and Barbados were regarded by contemporary observers such as LIGON and WINTHROP, as part of the same epidemic. It is reasonable, despite the absence of a description of the symptoms, to conclude that the infectious disease in Barbados was also yellow fever. In the same way the outbreak in Yucatan was undoubtedly yellow fever, and since the association of the islands with terra firma was very close, it is not unreasonable to conclude that the highly fatal disease in Cuba was also yellow fever. Owing to tides and winds it would be easy for a ship to convey infection from the Windward Islands to terra firma but difficult in the opposite direction.

In order to obtain light on the genesis of the outbreaks, it is necessary to review very briefly two possibilities: (1) That yellow fever was endemic in the New World; (2) that the disease was introduced from Africa.

The history of Yucatan and Havana before 1648 has been very fully described by CARTER (1931), and only a brief resumé need here be given.



*Yucatan.* Before the conquest of the Mayas the only reference in their chronicles to an epidemic disease is to the Maya Cimal which is supposed to have occurred in 1482 or 1483. No precise details are given of this disease. From the Spanish conquest to 1648 the only pestilence suggestive of yellow fever is that attacking the members of MONTEJO's expedition in 1527, although in the 120 years between 1527 and 1648 there had been an enormous influx of non-immunes from Spain. The only diseases recorded in this period are those well known in Europe, smallpox, measles and tarbardillo (probably typhus).

*Havana.* The island of Cuba was settled in 1511 by VELASQUEZ with 300 residents from Santo Domingo. From that date till 1648 no epidemic resembling yellow fever is recorded, with the exception of an outbreak in 1620 when an epidemic of pernicious fevers decimated Havana, lasting from June to November, and carrying away many victims both from the town and from the flota (PEZUELA, 1868-1878). CARTER (1931), quoting Dr. BEATO of Havana, shows that this fever did not excite sufficient alarm for rogations or processions. The evidence therefore is in favour of the view that yellow fever was not present in Cuba before 1648 or 1649. After this date it remained in the island till 1655, when it totally disappeared. In the meantime, in July and August of 1653, "the same fevers afflicted Santiago and Bayama as the capital had suffered three years ago, with equal havoc as in Havana" (PEZUELA, *Historia*, tom. ii, p. 112).

*Guadeloupe.* The only disease described by BRETON (1665) and DU TERTRE (1654 and 1667) as having occurred in Guadeloupe before 1648 is the coup de barre previously mentioned.

*St. Christopher.* Captain THOMAS WARNER, in 1620, accompanied ROGER NORTH to Guiana: on the voyage he learnt from a Captain PAINTON of the fertility of St. Christopher. In 1623, with thirteen others, he sailed for St. Christopher, landing there on 28th January, 1624. Caribs were then present in the island, while a year later French colonists arrived under d'ESNAMBUC and DU ROSSEY, who had procured from Cardinal RICHELIEU the privilege of establishing plantations in St. Christopher and Barbados. Apart from deficiencies of food on the voyages out—DU ROSSEY on the "Cardinal" had only sixteen out of seventy persons alive when he arrived at Pointe de Sable—the colonists thrived, though in 1629 both the French and English were temporarily driven out by an attack of the Spanish. Until 1647, however, there is no history of epidemic disease. Apart from the references to the epidemic in that year given in association with the Barbados outbreak there is no eyewitness account, but Père PELLEPRAT (1655) refers to an epidemic sickness in the island in 1652-3 which was communicable, fatal and general: this disease was associated with a shortage of food. Whether or not this infectious condition was the aftermath of the epidemic of 1647-1648 is uncertain.

*Barbados.* In 1625, JOHN POWELL the elder arrived in the "Olive," and took possession of the uninhabited island: eighty colonists landed in 1627 and by

1629 between 1,600 and 1,800 persons had arrived. Yams, cassava, Indian corn and plantains were obtained from Guiana, and for a time all went well. Sir HENRY COLT (1631), the first English tourist to visit the West Indies, has left a full account of the island in its early years. He was struck by the absence of disease but found the "aboundance of smale knatts by ye sea shore towards ye sunn goinge down" rather annoying. On the other hand, "noe body lice will increase beyond ye Tropick but head lice will, and ye Itch and scratchinge cannot be avoyded." The drunken habits of the colonists and their laziness, however, were the cause of their lack of food whenever for any cause their crops failed. The years 1630-1631 were, in fact, known as "the starving time," and whenever climatic conditions were unfavourable or communications with England failed the colonists were faced with want. The same conditions continued throughout the seventeenth century. For example, in May, 1666, FRANCIS LORD WILLOUGHBY wrote to KING CHARLES II, warning him that "Barbados and ye rest of ye Caribbee Islands belonging to yor Majesty have not clothes sufficient to hide their nakedness, nor food to fill their bellies." In 1673 "there is great want of Provisions on the Island," and, in 1674, "We have continued here in great peace and health but by reason of the Interruption of Trade by the present warr in greate want of all things but especially provisions." Apart from this lack of food there was no history of an epidemic outbreak in Barbados before 1647 or for some years after.

### THE WEST INDIES AND AFRICA.

The evidence thus shows that yellow fever was not endemic in any of the areas where it appeared in epidemic form in 1647-1649. If the disease was not endemic in the Caribbean region it must have been introduced from outside. There is considerable evidence to show that this area was West Africa. This view is not new, for CHARLES DE ROCHEFORT, writing in 1658, has the following suggestive statement, "The air of all these islands (the Caribbean) is quite temperate and remarkably healthy when one has become accustomed to it. The plague was formerly unknown there, just as in China and some other parts of the East. But some years ago the greater part of these islands were afflicted with malignant fevers that the doctors held to be contagious. This bad air had been carried by ships which came from the coast of Africa. But to-day one no longer hears such diseases spoken of."

If, as DE ROCHEFORT suggests, a contagious and malignant fever had been brought from Africa but had later disappeared, were there any circumstances which would favour the transmission of yellow fever from West Africa to Barbados and St. Christopher? In order to consider this question it is necessary to review very briefly the economic history of these islands. When first colonized the principal crop grown for export was tobacco. The population of Barbados increased rapidly from 6,000 in 1636 to, according to

one authority, 37,200 in 1643: "At the beginning, all the foreign Inhabitants of the Caribbees apply'd themselves wholly to the culture of Tobacco, whereby they made a shift to get a competent livelihood, but afterwards the abundance that was made bringing down the price of it" (DAVIES, 1666), other crops had to be grown. This other crop was sugar. In St. Christopher the English and French agreed in 1639 to cease planting for a year and in 1640-1642 the colonists turned entirely to the cultivation of sugar cane. Unfortunately, it was at once found, both in St. Christopher and Barbados, that white labourers were unsuited to raising sugar cane, and as a result the small holders were displaced, the work being done by negro slaves under the direction of a few white overseers. In 1667, JOHN SCOTT records that since 1643 no less than 12,000 good men had left Barbados for other plantations, while from 1645 to 1667 the owners of property changed from 11,200 small holders to 745 owners of large estates. Despite this exodus of small holders, however, there was a considerable number of new arrivals from England during the fifth decade. HARLOW (1926) states that as England became too hot for unrepentant royalists emigration to Barbados became the recognized course. Officers of the King's Army, if captured, preferred the option of going to Barbados to imprisonment in the Tower, and the same course was ruthlessly forced on the royalist rank and file to such an extent that the phrase 'to Barbados a man' became proverbial. These unfortunates were shipped over as indentured servants to a virtual slavery. The tide of immigration may be estimated by the fact that the white population of Barbados in 1645 was 18, to 20,000, and 5 years later, despite the epidemic of 1647-1649, had risen to over 30,000. At the same time the negro population increased by leaps and bounds. In 1640 there were but a few hundred negroes in Barbados, but as the cultivation of sugar cane increased so did the demand for negro slaves. In 1645, Sir GEORGE DOWNING wrote that the people of Barbados "have bought this year no less than a thousand negroes and the more they buie the more they are able to buye, for in a year and a halfe they will earne with God's blessing as much as they cost." In 1645 there were nearly 6,000 negro slaves in the island and five or six years later more than 20,000. The presence of these wild Africans caused some alarm, and to guard against sudden revolts most of the estate managers' houses were fortified and provided with large cisterns for the storage of water. As early as 1648 PETER FORCE reported many hundreds of escaped negro slaves in the woods. Most of these negroes came from the Gambia and Sierra Leone. An order from the directors of the English Guinea Corporation, ROWLAND WILSON and others, to one of their merchants, JAMES POPE, aboard the ship inappropriately named "Friendship," exhorts him (9th December, 1651) to exchange a cargo of spirits at the River Gambia for "as many lusty negroes or cattle as possible and send them to the Barbados" (Calendar of Duke of Portland's MSS.). The slaves were also transported in Dutch ships of from 100 to 200 tons to Barbados as well as to St. Christopher and Guadeloupe, where also the

Governor, the SIEUR DE HOULE, had introduced sugar planting in 1646. Between 1637 and 1642 the Dutch captured all the Portuguese posts on the African coast from Arguim in the far north down to St. Paul de Loanda, the capital of Angola, while from St. George d'Elmina, Cape Coast and Axim they dominated the Gold Coast against all other Europeans until about 1652 when the Portuguese regained many of their forts. Thus, during the fifth decade of the seventeenth century slaves were for the first time transported in large numbers in Dutch and English ships to the lesser Antilles. The passage from West Africa to Barbados was, however, much shorter than that to the mainland of America or to Santo Domingo, Cuba, and the other Spanish islands. The chances of transferring infected *Aedes aegypti* from Africa to Barbados were thus much greater than when the slavers' voyages were far longer. In addition, there was a greater possibility, since all African natives are by no means immune to yellow fever, of a patient, black or white, contracting the disease on board ship and actually arriving in the West Indies while still in the infective stage. Unfortunately, no figures are available for the death rates in Dutch ships during the period under discussion. The Royal African Company's ledgers, however, give details of six English slave ships between 1670 and 1683. Of 1,653 slaves, 185 or 11.2 per cent., died on the voyage; sometimes the mortality was greater. "The Lady Francis in 1681 had not above 20 or 30 negroes liveing of abt 160 taken in at the Bite." (Letter from Royal African Company's factor at Barbados.)

It is probable that a similar state of affairs occurred in the Dutch ships during the years 1640-1649: at the same time the presence of a large non-immune population in the lesser Antilles and the provision of water cisterns would supply conditions suitable for the occurrence of an epidemic of yellow fever.

#### CONCLUSIONS.

The occurrence of an epidemic resembling yellow fever in Barbados, St. Christopher, Guadeloupe, Yucatan and Cuba in the years 1647-1649 is discussed. Evidence is brought forward in favour of the view that this infection was carried in ships from West Africa to Barbados or St. Christopher in the year 1647, economic and political conditions at this time favouring the transmission of either infected mosquitoes or persons in the infectious stage of yellow fever.

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## TWO AUTOPSIES ON RHODESIENSE SLEEPING SICKNESS; VISCERAL LESIONS AND SIGNIFICANCE OF CHANGES IN CEREBROSPINAL FLUID.

BY

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The purpose of this paper is to describe postmortem examinations made on two cases of *rhodesiense* sleeping sickness, the points of especial interest being:—

- (1) No effective treatment to modify the natural development of the disease.
- (2) The marked visceral lesions.
- (3) The slightness of the lesions in the central nervous system and the correlation with the changes in the cerebrospinal fluid.
- (4) The concomitant infection with tuberculosis.

The cases were seen at Kahama which lies 200 miles south of Lake Victoria, in Tanganyika Territory, East Africa. Ten years ago there was a large epidemic of sleeping sickness in this area, and since then sporadic cases have occurred.

### DESCRIPTION OF CASES.

CASE 1. MABULA s/o MSALI. Aged ? 35. Admitted 1.11.38.

*History*, 6 months; headache and pain behind manubrium sterni. O.E. Emaciated man, weight 32 kg.; can walk, but with difficulty. Temperature, 101° F. Pulse 84. Liver and spleen moderately enlarged. Lymph nodes (1 cm.) palpable in groins, axillae, and epitrochlear region. L. hydrocele, 12 cm.; R. epididymis hard and nodular. Intelligence fair; no evidence of lesions of central nervous system. No oedema of legs. Blood, Hb, 65 per cent.; numerous trypanosomes. C.S.F.—tryps., 300/mm.<sup>3</sup>; cells, 14/mm.<sup>3</sup>; protein, 0.025 per cent.

Patient was treated with undecane diamidine for 4 days. The blood became free from trypanosomes but the C.S.F. was unchanged. 9.11.38. Trypanosomes reappeared in blood. Given Bayer 205, 1 gramme at noon, but died 11.30 p.m.

### POSTMORTEM (10 hours later).

Thyroid small and somewhat cystic.

*Thorax*.—Each pleura contained about  $\frac{1}{2}$  litre slightly turbid yellowish fluid with a few flakes of fibrin; dense adhesions round much of R. lung at side and back.

Lungs, R. upper lobe contained solid patches 2 to 5 cm. across with whitish nodules

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on sections; a few similar patches in L. upper lobe. On pleural surface of lower lobes there were tiny abscesses.

Pericardium, distended with about  $\frac{1}{2}$  litre of fluid like that in pleurae.

Heart, 170 gramme. Coronary vessels prominent. Myocardium brownish.

*Abdomen.*—About  $\frac{1}{2}$  to 1 litre of fluid like that in pleurae.

Stomach, mucosa atrophic; a few petechial submucosal haemorrhages.

Intestines, a few shallow ulcers in transverse colon.

Liver, 1,230 gramme. Cut surface olive-brown, and markings indistinct.

Spleen, 510 gramme. Densely adherent to surrounding structures and white fibrous patch on diaphragmatic surface. Substance very soft; purplish with large white dots.

Kidneys, 84 grammes. Genitals, small hydroceles.

*Lymph nodes.*—Cervical, slightly enlarged and red; one measured 1 cm. each way and on section it was mottled white and red. Bronchial, not obviously enlarged. Mesenteric, much swollen,  $2.5 \times 2 \times 2$  cm. Retroperitoneal, somewhat enlarged and whitish. Axillary and inguinal, slightly enlarged.

*Brain.*—Dura mater adherent over cerebral hemispheres. Abundance of slightly blood-stained cerebrospinal fluid. Subdural petechial haemorrhages over petrous bones and cavernous sinuses. Vessels of cortex rather distended and brain substance slightly congested. Petechial subpial haemorrhages in lower surface of L. cerebellar hemisphere. On cutting into the brain, small capillary haemorrhages were seen in a few places especially in R. occipital lobe.

*Smears.*

Pleural fluid—numerous cells, lymphocytes and also monocytes; disintegrating trypanosomes.

Pericardial fluid—numerous cells, mostly lymphocytes, also monocytes, endothelial cells and a few giant cells with 3 to 10 large round nuclei; trypanosomes present, many disintegrated.

Peritoneal fluid—cells less numerous, but as in pericardial fluid; trypanosomes present.

Cerebrospinal fluid—moderate numbers of monocytes and lymphocytes, many R.B.C., degenerating trypanosomes.

Hydrocele fluid—rare mononuclear cells.

### *Histology*

*Heart.*—Epicardium.—Much thickened in most places by cellular exudate, and in some parts by inflammatory oedema. The cells are mostly lymphocytes, but histiocytes, plasma cells and polymorphs are present in considerable numbers. The capillaries stand out prominently. There are also lymphatic spaces distended with endothelial cells and lymphocytes, and with a few polymorphs and plasma cells. The coronary vessels, where they run through the epicardial infiltration, are surrounded by a considerable adventitia, which is almost devoid of inflammatory cells; there is no evidence that the pericarditis is in any sense secondary to an arteritis or peri-arteritis. The surface epithelium appears to be lost. A striking feature is the complete absence of fibrinous exudate on the surface of the serous membrane, and there is only a minimal amount of fibrin deposition amongst the cellular exudate. With Giemsa, a few trypanosomes were seen in the superficial layers of the epicardium.

Endocardium.—The lesions vary greatly but on the whole they are much slighter than those of the epicardium. Some parts are free from inflammation; in other parts there are cells of the histiocytic type including many with large lobed nuclei, but both lymphocytes and polymorphs are relatively scanty; in some parts even although the endocardium is not thickened there is a considerable accumulation of polymorphs and lymphocytes. The endothelial lining is mostly intact.

Myocardium.—There is a general diffuse infiltration of the interstitial connective tissue with histiocytes, and also polymorphs, lymphocytes and plasma cells. In some parts these cells form collections between the muscle bundles. In some areas the muscle fibres are normal or even hypertrophied; but in most places they are shrunken and disintegrating, the fibres being separated by oedema and much brown pigment occurring. No tubercles are present.



FIG. 1.—Portion of epicardium and myocardium, Case I, showing cellular infiltration, etc.  
 $\times 67$ .



FIG. 2.—Epicardium and myocardium of monkey, infected with *T. rhodesiense*, killed after 83 days; for comparison with Fig. 1.  $\times 110$ .



FIG. 3.—Myocardium, Case I, to show the infiltration of cells between the muscle fibres.  
 $\times 240$ .

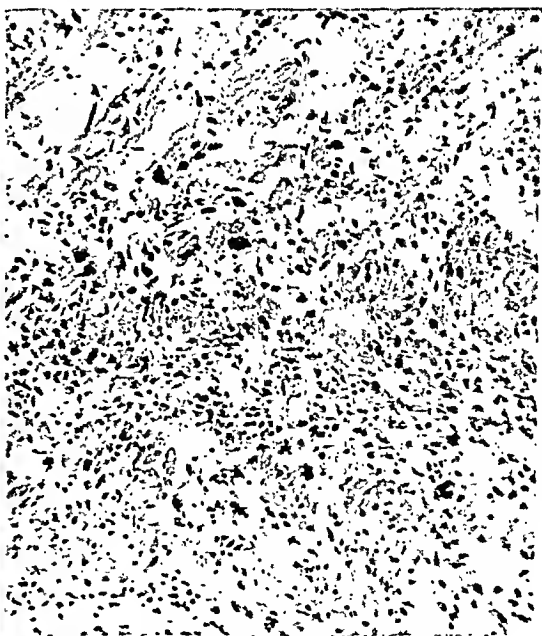


Fig. 4.—Myocardium, Case I, to show infiltration of cells, destruction of muscle fibres and sclerosis.  $\times 110$ .



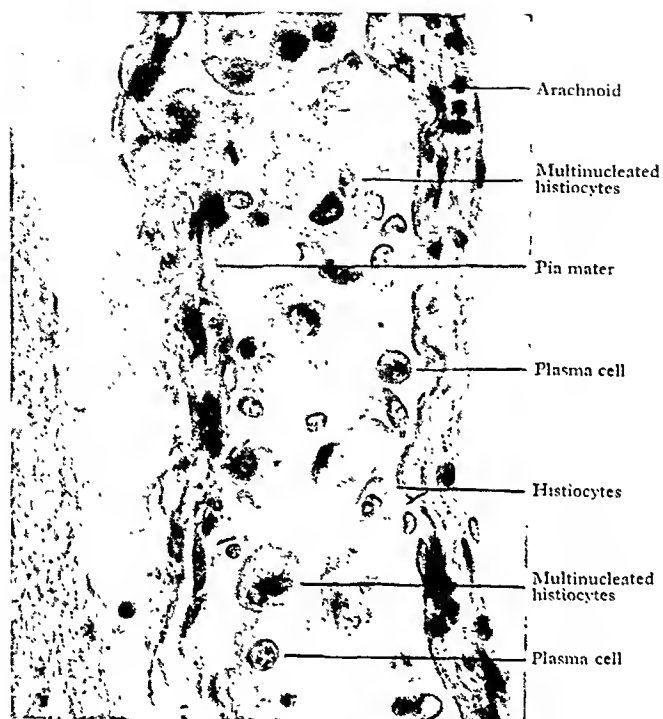


Fig. 5.—Meninges, Case I, to show the cellular exudate.  $\times 600$ .

*Lungs*.—Numerous small tubercles and centres of caseating pneumonia are present. Giant cells few; little or no fibrosis.

*Liver*.—Numerous foci of caseation, which tend to become confluent; in other parts dense collections of medium sized mononuclears. Elsewhere the hepatic cells are small and shrunken, the normal architecture of the liver being obscured; many cells contain brownish pigment.

*Stomach*.—Mucosa slightly atrophic.

*Large intestine*.—Ulcers in mucosa with necrotic base, but no evidence of tuberculosis.

*Spleen*.—Large areas of necrosis and caseation, and also scattered smaller tubercles. Elsewhere, Malpighian bodies small and pulp is poor in cells, many of which seem necrotic. There are a few cells containing 2 to 3 large nuclei, and also phagocytes laden with brown pigment.

*Lymph Glands*.—Some are completely caseated. Others contain caseating tubercles. In others, the lymphoid tissue of cells is depleted and the channels are distended with fluid, the reticulo-endothelial cells being prominent. Some glands contain phagocytes laden with brown pigment, others are fibrotic.

*Kidneys*.—Cells of convoluted tubes swollen and degenerate.

*Testes*.—Marked atrophy of seminiferous tubules and increase of connective tissue.

*Thyroid*.—Epithelium atrophic.

*Adrenal*.—Many strands of cells in cortex completely atrophic. Other organs approximately normal.

#### *Histology of Central Nervous System.* (By J. G. G.).

*Membranes*.—In many places the sub-arachnoid space over the upper cervical cord, the cerebellum and the cerebral hemispheres was infiltrated with cells of three kinds. By far the most abundant were histiocytes which varied in size from that of the monocytes of the blood to large bi-nucleated or multi-nucleated cells measuring 30 to 40  $\mu$  in diameter. In the larger cells the cytoplasm was usually irregularly rounded and granular and often it contained vacuoles in some of which the remains of red blood corpuscles could be seen. Many medium sized cells were elongated. The nucleus was usually vesicular with fine chromatin dots scattered through it. In the smaller cells it was reniform, lobed or horseshoe shaped. In the larger cells the nuclei were usually more rounded and many were irregularly indented. These cells so dominated the picture that the plasma cells and lymphocytes appeared very scanty. Most of the plasma cells were of the usual type but there were also cells with the typical clock-face nucleus and basophil cytoplasm, but of larger size, and in some of these the cytoplasm contained fine granules or vacuoles and the nucleus was reniform and appeared to be pushed against the cell membrane; these cells thus resembled to some extent the morular cells found in cases of sleeping sickness due to *T. gambiense*. While the infiltration was fairly widespread over the cortex, in many places forming a fairly thick layer, it did not tend to pass into the sulci either of the cerebrum or cerebellum as it does in general paralysis; but small collections of histiocytes were seen deep in the sulci in some places. The plasma cells and lymphocytes were most numerous immediately around the vessels. Some of the vessels on the surface of the medulla were infiltrated by such cells although histiocytes were not found in this situation. Among the infiltrating cells were to be seen clusters of granular material which stained fairly darkly with toluidin blue and appeared to be of a lipid nature. Among the thicker collections of cells fibrin threads could be seen. Slight infiltration either with lymphocytes and plasma cells or with histiocytes occurred along a few of the vessels penetrating from the meninges; some small vessels under the ependyma of the third ventricle were similarly infiltrated. The ependyma itself was normal. Slight infiltration with lymphocytes and plasma cells was also seen round the optic chiasma.

*Vessels*.—Round the cortical vessels there were often a few lymphocytes but real perivascular infiltration was absent. In the occipital lobe there were a few small petechial haemorrhages and at one place in the parietal lobe there was some brown pigment suggesting an old haemorrhage. The lumen of the vessels was often filled with masses of bacteria, presumably a postmortem growth.

*Nervous Tissue*.—Everywhere approximately normal.

*Choroid Plexus.*—In a few places there was slight infiltration with large cells, but most parts were altogether unaffected. Numerous corpora amylacea were seen in the choroid plexus as well as in the medulla and spinal cord.

*Eye.*—Retina normal; no exudate in sheaths of optic nerve.

*Trypanosomes.*—Not seen. (The postmortem changes were sufficient to cause degeneration.)

*Summary (Central nervous system).*—The type of infiltration found in the meninges was unusual in the predominance of histiocytes, and the comparative scarcity of lymphocytes and plasma cells. However, a few cells which resembled to some extent the "morula cells" characteristic of African sleeping sickness, were seen. The nervous system itself was approximately normal, in spite of the numerous trypanosomes in the cerebrospinal fluid. The comparative slightness of the meningeal reaction is in accordance with the low figures for cells and protein content in the cerebrospinal fluid.

### *Summary of pathological changes.*

Trypanosomal septicaemia.

Inflammatory effusions containing trypanosomes in pleura, pericardium and peritoneum.

Subacute inflammation of heart, principally myocardium and epicardium.

Numerous trypanosomes in cerebrospinal fluid, but the tissues of the nervous system were practically normal and the meninges were only slightly involved.

Tubercular consolidation of parts of both lungs, and tubercular foci in the mesenteric and other lymph nodes and in the liver and spleen.

N.B.—In an earlier paper (HAWKING, 1940) experiments are described on the strain of trypanosomes isolated from this case. The parasites were resistant to normal human serum, but this property was lost after seven passages through rats. The virulence of the strain for rats and its sensitivity to trypanamide *in vivo* were similar to those of other strains of *T. rhodesiense* in this region. Experiments *in vitro* suggested that the plasma of this case was somewhat deficient in trypanocidal power.

CASE 2. DOTTO s/o TUPA. Aged ? 55. Admitted 29.12.38.

*History*, 4 months; pains in head, thorax, abdomen, and legs. O.E. Very emaciated old man; stands and walks with great difficulty. Pulse 112, small volume; rales in chest. Liver enlarged. Marked oedema of ankles. Small lymph nodes in cervical, axillary, epitroclear and inguinal regions. Dementia marked. Limbs are plastic, maintaining any imposed posture, but not spastic. Blood, Hb. 80 per cent.; numerous trypanosomes. C.S.F.—tryps., 200/mm.<sup>3</sup>; cells, 10/mm.<sup>3</sup>; protein 0.028 per cent. Patient was given Bayer 205 1 gm. 29.12.38, but died 2.1.39.

### POSTMORTEM (1½ hours later).

*Thorax.*—Pleurae each contained about 300 c.c. of clear fluid; a few adhesions present.

*Lungs.*—Consolidation of middle part of R. lung (patient died lying on R. side); cut surface dark red; pieces sink in water. L. lung emphysematous.

Pericardium, about 500 c.c. clear fluid.

Heart, 203 gm. soft and flabby.

Aorta, a few streaks of atheroma.

*Abdomen.*—About 400 c.c. clear fluid.

Stomach, a few submucosal petechiae.

Liver, 1,860 gm. surface smooth, brownish red.

Spleen, 460 gm. dark red.

Kidneys, 116 and 174 gm.

*Lymph nodes*.—Cervical, axillary, and bronchial, small. Those of mesentery and along portal vein slightly enlarged and reddish, forming chains.

*Brain*.—Convulsions rather shrunk.

#### *Smears.*

Pleural fluid—numerous lymphocytes and monocytes, less numerous polymorphs and erythrocytes. Trypanosomes present in great tangled masses.

Pericardial fluid—numerous lymphocytes, monocytes, and endothelial cells; moderate numbers of degenerate trypanosomes.

Peritoneal fluid—Lymphocytes and endothelial cells; moderate numbers of degenerate trypanosomes.

Blood from jugular vein—numerous trypanosomes, mostly degenerate.

C.S.F.—Numerous lymphocytes, monocytes and trypanosomes.

The films also contained a few cocci which seemed to have no significance; degeneration of trypanosomes was due to postmortem change.

#### *Histology.*

*Heart*.—Epicardium is thickened but the cellular exudate is less abundant than in the first case, oedema being more marked. The cellular exudate consists largely of histiocytes. The surface epithelium is lost but there is no suggestion of a fibrinous exudate. In one area there is a granulomatous lesion which is probably tuberculous.

*Endocardium*.—The lesions vary greatly in different places but generally they are less than those of the epicardium. Some parts are comparatively normal; in other parts there is much thickening and infiltration with histiocytes, lymphocytes and plasma cells. In some parts there are considerable numbers of polymorphs and fibroblasts.

*Myocardium*.—The general appearances are like those of Case I, but the fibrosis is greater and the cellular infiltration somewhat less. The interstitial connective tissue is much increased and in some parts it contains fusiform collections of cells with large deeply staining angular nuclei resembling those of an Aschoff nodule. (These collections might be tuberculous in origin.) The muscle fibres appear mostly normal but in some parts they are shrunk and degenerate, mononuclear cells being present between the muscle fibres.

*Lungs*.—In places, the alveoli are filled with fluid and a few cells; some early caseation.

*Liver*.—The fibrous tissue round the portal sheaths is increased and contains collections of lymphocytes. The liver cells are slightly shrunk but otherwise normal. There are a few scattered small areas of caseation.

*Stomach*.—Mucosa atrophic.

*Spleen*.—Many small tubercles and foci of caseation; no giant cells. Malpighian bodies small, cells of pulp are mostly erythrocytes; much brownish pigment.

*Lymph nodes*.—In some, the lymphoid tissue is atrophic and contains only a few cells, while the lymph channels are distended with fluid and contain many large phagocytic cells with 1-3 nuclei. Other nodes contain small tubercles; some nodes are completely caseated. A section of one lymph node contained a male *W. bancrofti*.

*Testes*.—Atrophy of seminiferous tubules.

*Bone Marrow (rib)*.—Moderately extensive areas of atrophy and gelatinous degeneration.

*Pituitary*.—Connective tissue of anterior lobe much increased in amount; vesicles with colloid prominent. Other organs approximately normal.

#### *Histology of Central Nervous System. (J. G. G.)*

*Meninges*.—The meningeal infiltration in this case had the same general characters as that in Case I, but was less extensive and intense. Infiltrating cells were practically absent from the sulci, both of the cerebrum and cerebellum, and over many areas of the cortex no infiltrating cells could be seen. Over the cerebellum the infiltration was everywhere quite slight. Over the cerebrum it showed the same preponderance of large histiocytic cells as was found in Case I.

*Vessels.*—The small meningeal and intra-cortical vessels showed some collagenous thickening of their walls; and in the basal ganglia many vessels of medium calibre and a number of capillaries showed incrustation of their walls with material which stained darkly with haematoxylin (probably both calcium and iron). This was similar to the incrustation commonly seen in this situation in European brains.

*Brain Tissue.*—The neurons were approximately normal or only slightly degenerate. Corpora amylacea were numerous in the olfactory lobe, cerebellum, pons, upper cervical segments and choroid plexus.

*Choroid Plexus.*—Most of the plexus consisted of rather swollen connective tissue lightly infiltrated with cells, but at one place there was a dense collection of mononuclear cells of all kinds, together with a fibrinous exudate containing elongated cells.

*Trypanosomes.*—Two trypanosomes were seen in exudate over the cerebellum; elsewhere, in the membranes of the cerebrum and cerebellum small granules were seen which might represent trypanosome nuclei. The scarcity of trypanosomes is probably due to rapid postmortem degeneration.

*Summary (Central nervous system).*—In view of the large number of trypanosomes in the cerebrospinal fluid, the histological changes in the nervous tissue are remarkably slight. This, however, is in accordance with the low figures for the cells and protein content.

#### *Summary of pathological changes.*

Trypanosomal septicaemia.

Inflammatory effusions, containing trypanosomes in pleurae, pericardium and peritoneum.

Subacute inflammation of heart, principally myocardium and epicardium. Numerous trypanosomes in C.S.F.; but tissues and membranes of brain only slightly involved.

Tubercular foci in lungs, liver, spleen and lymph nodes.

#### DISCUSSION.

As indicated by the summaries of the postmortem and histological findings, the two cases here described are closely similar and they will be discussed together.

##### 1. EFFECT OF TREATMENT DURING LIFE.

Owing to the great improvement in therapy and in medical administration, opportunities for postmortem examination of untreated cases of sleeping sickness are fortunately now rare. Either the patient is cured at an early stage, or his life is much prolonged by successive courses of treatment, thus altering the lesions finally observed. In the two present cases the periods of treatment which were received (8 and 4 days respectively) were too brief materially to modify the progress of the disease and they may be disregarded.

##### 2. RELATION OF THE TRYPANOSOMIASIS AND THE TUBERCULOSIS.

These patients probably died of their trypanosomiasis before they died of their tuberculosis, but since the two infections presumably reinforced one another, it is needless to speculate on the share played by each in producing

the fatal issue. More important is the question as to which infection produced the lesions of the heart and the serous effusions. As regards the heart, the histological appearances were much like those produced by trypanosomes in monkeys, while no suggestion of giant cells or organised tubercles was discovered in the heart of either case during prolonged search. ASCHOFF (1936) states that tuberculosis in the pericardium is not common and in the heart it is rare, and that it can be recognised by the usual histological features. As regards the serous effusions, they contained trypanosomes in great numbers and they were closely similar to the effusions which are produced by trypanosomes in monkeys; no evidence of tuberculosis involving the linings of the serous cavities was seen by the naked eye, or histologically. Moreover, the occurrence of both myocarditis and serositis during autopsies on sleeping sickness cases is recorded by the early workers (see next paragraph). Consequently it is concluded that both the serous effusions and the heart lesions were due mainly to trypanosomiasis. It is possible, however, that the focus of inflammation recorded in the choroid plexus of Case 2 may have been tuberculous in origin.

The occurrence of tuberculosis in these patients was unexpected, and it is not clear how far it is an indication of the local prevalence of the disease in this region. In 1928, in the hospitals of the Kahama district, there were seventy-two patients with a diagnosis of "pulmonary disease" out of a total of 1,031 in-patients; it was noted that pulmonary tuberculosis was rare. (*Tanganyika Med. Rept.*, 1928.) The region (area 7,000 sq. miles) has a population of 80,000 unevenly distributed; moderate numbers of cattle are kept in the clearings. In the post-war decade there was some gold mining in the neighbourhood but, at the present time, contact with Europeans is not close.

### 3. VISCERAL LESIONS OF TRYPANOSOMIASIS.

Earlier workers on sleeping sickness concentrated their attention mostly on the changes in the blood, lymphatic organs and central nervous system; they were dealing primarily with *gambiense* cases, in which the disease is more chronic and the nervous symptoms predominate. Nevertheless, on turning up the accounts of their postmortem examinations, it is found that lymphocytic infiltration of the heart and effusions into the pericardium and other serous cavities are duly recorded by LOW and CASTELLANI (1903) and by BREINL (1905).

PERUZZI (1928) drew attention to the occurrence of myocarditis serositis and nephritis in monkeys infected with virulent strains of *T. rhodesiense*, and his findings have been corroborated in animals by subsequent workers; so far as is known, however, apart from sclerotic lesions in the hearts of two long-standing cases of *gambiense* infection, described by LAVIER and LEROUX (1939), no observations have been recorded of similar lesions occurring in man, presumably owing to the rarity of autopsies on untreated patients. In the present cases, the main visceral lesions due to trypanosomiasis were (a) the

changes in the heart which have been described above, and (b) effusions containing numerous trypanosomes in the serous cavities.

(a) The *heart lesions* indicate a subacute inflammation of the interstitial connective tissue of the myocardium and of its internal and external coverings similar to that of PERUZZI's Monkey 126. Through the kindness of Dr. J. F. CORSON, it was possible to examine monkeys infected with trypanosomes for various periods. Those killed after 80 days showed changes in the myocardium similar to the ones described by PERUZZI in his earlier cases, *viz.*, large dense infiltrations of monocytes, lymphocytes, and plasma cells between the muscle fibres, and under the epicardium and endocardium. In these monkeys smears of the pericardial fluid showed numerous parasites, but when the heart was fixed in formalin (within 15 minutes of the death of the animal) and the material was brought home to England and prepared for histological examination, it was very difficult to distinguish complete trypanosomes in the site where they had certainly been originally present. Consequently, the difficulty of finding trypanosomes in the human hearts is probably due to disintegration of the protozoa during postmortem changes and subsequent handling, rather than to their true absence.

(b) The *serous effusions* were closely similar to those described by PERUZZI; and similar effusions occurred in the monkeys killed by Dr. CORSON at Tinde. As in PERUZZI's monkeys, the cells of the effusions in these two autopsies were predominantly mononuclears. BURKE-GAFFNEY (1936) describes a child infected with *T. rhodesiense* and *W. bancrofti* in whom there was ascites containing great numbers of trypanosomes and mononuclear cells; this was interpreted as multiplication of trypanosomes in ascites due to other causes, but in view of the present autopsies and of PERUZZI's findings, the writer considers it as more probably another case of effusion due to trypanosomes.

It is probable that in acute cases of *rhodesiense* sleeping sickness the visceral lesions are much more dangerous to the patient than are those of the nervous system, especially since the nervous lesions are often slight, as in the present cases.

#### 4. CHANGES IN THE CENTRAL NERVOUS SYSTEM AND THEIR RELATION TO THOSE IN THE CEREBROSPINAL FLUID.

In these two cases, the histological changes in the brain were slight, being confined to slight oedema and cell infiltration of the membranes and choroid plexus, plus the small focus of inflammation found in the choroid plexus of Case 2. The changes in the C.S.F. consisted of great numbers of trypanosomes but only slight increase of the cells and protein content. It is usually believed that the cells and protein of the C.S.F. are a more reliable guide to the extent of the brain lesions than is the presence or absence of trypanosomes; and in these cases the smallness of the changes in the cells and protein would be interpreted as an indication that the nervous system was not yet much involved. This interpretation is confirmed by the pathological findings. PERUZZI concluded from observations on monkeys that although trypanosomes may reach

the C.S.F. in great numbers in the early stages of the disease, they cannot survive there until the protein content of the fluid is appreciably increased. The present findings support that conclusion and so do certain experiments on the survival of trypanosomes *in vitro*. YORKE, ADAMS and MURGATROYD (1930) found that trypanosomes suspended in a mixture of Locke's solution and serum at 37° C. survived for over 24 hours, but if suspended in pure Locke's solution they died in a few minutes. Judging by their figures and by the writer's own experience, trypanosomes will not survive *in vitro* for 24 hours at 37° C. unless at least 1 part of serum is present to 15 parts of Locke's solution, *i.e.*, about 0.4 per cent. protein. On a number of occasions during the work at Kahama, the writer added small inocula of trypanosomes to specimens of C.S.F.

TABLE

THE FAILURE OF TRYPANOSOMES TO SURVIVE IN CEREBROSPINAL FLUID INCUBATED AT 37° C. AND THE PROLONGATION OF LIFE WHEN SERUM WAS ADDED.

The C.S.F. contained trypanosomes 4 per mm<sup>3</sup>, cells (some morular) 120 mm.<sup>3</sup>, and protein 38 mg. per 100 ml. It was withdrawn  $\frac{1}{2}$  hour before the experiment began and the trypanosomes were concentrated by centrifuging. Tube 1 was filled with the concentrated C.S.F., tubes 2 and 3 by mixtures of this with serum and Locke-glucose.

Tube.	Contents of Tube.	Number of living trypanosomes per mm <sup>3</sup> .			
		Start.	2 Hours.	7 Hours.	24 Hours.
1	Pure C.S.F. ... ..	70	2	0*	
2	C.S.F. + Locke-glucose + human serum, equal parts ... ..	50	45	15	0*
3	C.S.F. + Locke-glucose + sheep serum, equal parts ... ..	(50)	15	5	0*

\* Each tube inoculated into one rat; no infection produced, although rats inoculated with blood from this patient became infected after an incubation period of 5 days.

from sleeping sickness patients. The protein content of the specimens (including serum carried over with the inoculation) averaged about 0.1 to 1.15 per cent. In these fluids at 37° C. the trypanosomes died in 2 to 4 hours. By contrast, if they were suspended in equal parts of serum and C.S.F. (*i.e.*, protein 3.5 per cent.) they survived over 24 hours. When human blood naturally containing trypanosomes is incubated at 37° C., living trypanosomes can be found in the serum over the clot 24 hours later; but if cerebrospinal fluid naturally containing trypanosomes is similarly incubated, the parasites die in less than 1 to 2 hours. (Experiment repeated on five occasions when suitable patients were available.) The addition of serum to the fluid prolongs the life of the organisms considerably. The accompanying table shows a typical protocol;



(incidentally, this experiment demonstrates that trypanosomes of the *T. rhodesiense* type from the C.S.F. survive as well in normal human serum as they do in sheep serum, *i.e.*, that they are serum-resistant.) All these facts furnish strong support to the view that it is not possible to have a true invasion of the cerebrospinal fluid by trypanosomes so long as the protein content is low, and that such individuals as are found therein during the early stages of the disease have been washed out from some focus elsewhere. On the other hand, it must be admitted that when trypanosomes are found in the fluid withdrawn by lumbar puncture, all the organisms appear active and that degenerate forms are very rarely seen. In the present cases, the great number of parasites in the cerebrospinal fluid was probably a terminal event.

#### SUMMARY.

Two autopsies are described of cases of *rhodesiense* sleeping-sickness, in which no appreciable treatment had been received. There were extensive trypanosomal effusions in the pleural, peritoneal and pericardial cavities, and marked inflammation of the myocardium, epicardium and endocardium. These visceral lesions were closely similar to those described by PERUZZI in the trypanosomiasis of monkeys. The cerebrospinal fluids of these cases contained numerous trypanosomes but the cell-count and protein content were not much raised; corresponding to this pathological pattern in the fluid, the histological lesions in the central nervous system were very slight, being limited to a histiocytic infiltration of the cerebral membranes. From these cases and from experiments *in vitro*, it is concluded that it is not possible to have a true invasion of the cerebrospinal fluid by trypanosomes until the protein content is considerably increased. In the absence of treatment, the visceral lesions of *rhodesiense* sleeping-sickness are probably more often fatal than the lesions of the nervous system.

These two cases also suffered from widespread infection with tuberculosis.

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## IMMUNIZATION AGAINST BOVINE TRYPANOSOMIASIS.

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Professor CLAUS SCHILLING (1936) has summarized the results to that date of an experimental attempt to prove his contentions "(1) That young animals (foals, calves) in the first weeks of their life possess a considerable resistance against the African trypanosomes transmissible by tsetse flies; (2) That this natural resistance can be enhanced to a full immunity against superinfections by the fly either by minimal infections or by preventive vaccine treatment."

He writes: "In a tsetse-free area in Tinde, I treated prophylactically thirty-eight calves, and at the end of June, 1934, together with their mothers and ten controls, I sent them to Masumbwe, a place where all the cattle introduced previously had died from nagana. I have examined them once a week until November, 1934; after my departure the Director of Veterinary Services, Mr. H. E. HORNBY, took charge of the supervision and the examination of blood-slides once a month and reported to me regularly until the end of July, 1936. I express my sincere thanks to Mr. HORNBY and Mr. R. L. CORNELL for this quite objective control. The time of two years is sufficiently long to allow the following conclusions: . . . "

These conclusions are too lengthy to reproduce in full, but are based on evidence of survival at the end of July, 1936, of three (25 per cent.) of twelve control calves; four (44 per cent.) of nine vaccinated calves, and eight (47 per cent.) of seventeen calves treated with minimal infective doses. From these figures SCHILLING deduced a measure of natural resistance in young calves that could be enhanced by vaccination with dead trypanosomes or by setting up a primary infection with a very small number of living trypanosomes. The point of importance is that he believed the survivors to be now immune in the way that most large African game animals within tsetse-fly belts are immune. They could thus be maintained in the presence of tsetse, and their calves would be very good subjects for immunization, even if the fact of their being born of immune parents and subjected to natural infection at a very early age was not, by itself, enough to permit their survival.

In view of these widely published high hopes, the subsequent history of the little herd is of interest.

At the end of 1936 the experimental calves had become 2-year-old heifers and bulls. They numbered sixteen (not fifteen, as might be inferred from SCHILLING's article) and, according to my own notes, three of them were controls, four had been vaccinated with dead *T. brucei* and *T. congolense*, and nine had received minimal infecting doses of *T. brucei*, *T. congolense* and *T. vivax*. Full details are given in a series of articles on the experiment in the *Zeitschrift für Immunitätsforschung*, LXXXIII, LXXXV, LXXXVII and LXXXVIII. In the same herd were five cows—the remnant of the mothers of these experimental animals—and three calves, born recently. They were maintained at Masumbwe, a village north of Tabora, in what was formerly a sleeping-sickness area of the Western Province of Tanganyika. I inspected them on 3rd December, 1936, and my findings are condensed into Table I (p. 169).

At this time Masumbwe was at its best. A comparatively short dry season had come to an end in November and there was abundance of grazing. In a short visit I could not determine how frequently the cattle were bitten by tsetse: I saw none near the herd, though two or three were caught on my lorry before I arrived at their camp. The general appearance of the animals was excellent (Fig. 1) and anyone who saw them and was told that they had been living in "fly" for more than 2 years was bound to conclude (1) that they were immune to a degree not far removed from that of game animals; (2) that some—cows and controls—had developed this through a natural fitness to survive, and (3) Professor SCHILLING's treatment had contributed to the survival of the others.

But I was not satisfied that the animals had been exposed to continual infection. I knew from experience that a considerable percentage of cattle of all ages recover from an infection which may result from the bite of a single tsetse, but that very few indeed can stand up to daily attacks by fly. It seemed unlikely that as many as five cows out of less than forty should have achieved



FIG. 1.—Part of the herd in December, 1936.



FIG. 2.—The whole herd in January, 1941.



immunity through mere exposure to continual infection. I planned, therefore, to move the herd to an area where I knew they would be exposed to such infection.

TABLE I.

COMPOSITION OF EXPERIMENTAL HERD ON 3RD DECEMBER, 1936.

Animal's No. and description.	Treatment.	Condition.	Hb. by Tallqvist.	Blood-smear.
<i>Two-year-olds.</i>				
3. F. Black ... ..	Vaccinated	Good	60	T.c.+
4. F. " ... ..	Control	"	80	N.T.S.
5. F. " ... ..	M.I.D.	"	70	"
8. M. Black ... ..	"	"	70	T.c.+
9. M. " with star ... ..	"	"	75	N.T.S.
12. M. Brown ... ..	Vaccinated	Very good	75	"
17. M. Black ... ..	"	Good	65	T.c.+
18. F. Black with white belly and brush	Control	"	70	N.T.S.
19. M. " with light strip along back	M.I.D.	"	65	"
21. M. " and white ... ..	"	"	70	"
25. F. " ... ..	"	"	75	"
27. F. " with white brush ... ..	"	"	70	T.c.+
35. F. " In-calf ... ..	"	"	75	N.T.S.
40. F. Brown ... ..	"	"	70	"
44. M. Black and white ... ..	Vaccinated	"	70	"
61. M. " ... ..	Control	Very good	70	"
<i>Cows.</i>				
12. Grey. Tail without brush ...	No treatment	Poor but improving	60	T.c.+
89. Brown. In-calf ... ..	"	Very good	90	N.T.S.
214. Red and white. Calved 16.9.36 ...	"	Poor	60	"
219. Black. Aged. Calved 28.8.36 ...	"	Good	75	T.c.+
226. Black. Tip off right ear. Calved 8.9.36 ... ..	"	"	70	N.T.S.
<i>Calves.</i>				
62. F. Brown, with grey neck. From cow 219. Born 28.8.36 ... ..	"	"	85	"
63. F. Brown, with star and white belly. From cow 226, on 8.9.36 ... ..	"	"	90	"
64. Black and white. From cow 214, on 16.9.36 ... ..	"	"	90	"

M.I.D. = minimal infective doses. T.c.+ = *Trypanosoma congolense*.

N.T.S. = No trypanosomes seen.

Before this was done, misfortune fell on the herd. On 10th June, 1937, I received a telegram informing me that seven cattle had died and many others were sick. An Assistant Livestock Officer, Mr. J. W. T. HOLLOWAY, who



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9. M. " with star ... ..	"	"	75	N.T.S.
12. M. Brown ... ..	Vaccinated	Very good	75	"
17. M. Black ... ..	"	Good	65	<i>T.c.</i> +
18. F. Black with white belly and brush	Control	"	70	N.T.S.
19. M. " with light strip along back	M.I.D.	"	65	"
21. M. " and white ... ..	"	"	70	"
25. F. " ... ..	"	"	75	"
27. F. " with white brush ... ..	"	"	70	<i>T.c.</i> +
35. F. " . In-calf ... ..	"	"	75	N.T.S.
40. F. Brown ... ..	"	"	70	"
44. M. Black and white ... ..	Vaccinated	"	70	"
61. M. " ... ..	Control	Very good	70	"
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62. F. Brown, with grey neck. From cow 219. Born 28.8.36 ... ..	"	"	85	"
63. F. Brown, with star and white belly. From cow 226, on 8.9.36 ... ..	"	"	90	"
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Before this was done, misfortune fell on the herd. On 10th June, 1937, I received a telegram informing me that seven cattle had died and many others were sick. An Assistant Livestock Officer, Mr. J. W. T. HOLLOWAY, who



investigated the outbreak at once, reported that the dead animals were Oxen Nos. 12 and 17, Heifer No. 27, Cows Nos. 12 and 214, and Calves Nos. 62 and 64. Of these seven, blood smears were received from four—Oxen Nos. 12 and 17, Cow No. 214 and Calf No. 62, and all were positive for *T. congolense*. Of the remaining seventeen (for Cow No. 89 had calved since I saw the herd in July, 1936), several appeared off colour, but in the blood smears of only three (Heifer No. 3, Ox No. 9 and Calf No. 63) were trypanosomes (*T. congolense*) found. I concluded that during the past three years the herd had not been exposed to frequent attacks by tsetse, that recently there had been such an attack, and this, in the case of the animals which died, had broken down the defence of partial immunity engendered by a previous infection.

Shortly afterwards, on 26th June, a Veterinary Officer, Mr. M. A. MOLLOY, reported on the eighteen survivors. He said that eight were definitely off colour, presenting the characteristic appearance of fly-struck animals. He failed to find any tsetse, and wrote: "The past history and all the evidence I could collect point to the fact that even to date the herd has not been exposed to frequent attacks by tsetse, so I would suggest for your consideration that mechanical transmission and the inclement, really cold, weather conditions extant are factors which are playing an important part in the breaking down of the partial immunity engendered by previous infection."

I should state here that among the seven animals which died, two, Calves Nos. 62 and 64, had been infected deliberately on 28th January, 1937. On that date the Veterinary Officer had drawn blood from the mother (then *T.c.* +) of one of the three calves Nos. 62, 63 and 64, and inoculated them with this blood. All showed trypanosomes soon afterwards; No. 62 and 64 both died on 20th May, and No. 63 on 29th June. Incidentally, at the time of the inoculation he castrated all but one of the experimental males.

By September, 1937, the herd had picked up again, and Mr. MOLLOY wrote that on 9th September, Heifer No. 3 was the only animal off colour; all the rest were in very good condition. Still no tsetse were seen. Smears taken every month showed that most of the animals were in a state of premunition regarding one or more species of trypanosomes, as usually two or three smears from animals, which differed from month to month, were positive for trypanosomes, the species of which could not be determined with certainty by the native microscopist who reported on them.

It was not until July, 1938, that the herd was moved into unmistakable tsetse country. At the end of this month they travelled more than 100 miles to the Tsetse Research Camp of Dr. C. H. N. JACKSON, at Kakoma, south of Tabora, and thereafter were exposed definitely to daily bites by tsetse. At this time the herd consisted of twenty animals: Bull No. 61; Oxen Nos. 8, 9, 19 and 44; Heifers Nos. 3, 4, 5, 18, 25, 35 and 40; Cows Nos. 219 and 226, and Calves Nos. 65, 66, 67, 68, 69 and 70. This means that since the tbreak recorded above, one ox (No. 21) and one cow (No. 89) had died, and

Calves Nos. 66-70 had been born to Dams Nos. 226, 4, 35, 219 and 40 respectively. Of the two deaths, that of No. 21 was almost certainly due to *T. congolense* infection; the cause of the death of Cow No. 89 was not ascertained. Just before they moved from Masumbwe, the number of positive smears among those taken from each animal once a month went up, reminding one of the outbreak of a year previously. The numbers of the animals which yielded positive blood smears between September, 1937, and June, 1938, are given in Table II.

TABLE II.

Month.	Animals positive for trypanosomes.
1937	
September	Nos. 3, 25
October...	Nos. 3, 25, 40
November	Nil
December	Nos. 19, 25
1938	
January...	Nil
February	Nos. 8, 9, 25, 89
March ...	No. 226
April ...	Nos. 5, 68
May ...	Nos. 8, 9, 40, 44, 65, 67
June ...	Nos. 21, 40, 65, 67, 68, 219, 226

Calf 65 was born on 20th May, 1937, Calf 67 on 12th October, 1937, and Calf 68 on 7th October, 1937, and the fact that all were showing trypanosomes in the first half of 1938 is strong evidence of the occurrence of some tsetse at Masumbwe at this time.

Soon after the herd's arrival at Kakoma in August, 1938, the return of positive smears became higher than ever before. This is seen from Table III.

In November, 1938, Heifer No. 4 died; in January, 1939, Bull No. 61 died, and in February, Heifers Nos. 25 and 35. All showed trypanosomes in their blood at the time of death, and it is reasonable to attribute their loss to trypanosomiasis. In spite of the evidence of active disease furnished by these deaths and by the large number of animals showing trypanosomes, the general appearance of the herd was by no means bad when I saw it on 4th March, 1939, and it was even furnishing a little milk.

However, deaths continued to occur: In May, 1939, Heifer No. 5; in June, Ox No. 9 and Heifer No. 40; October or November, Cow No. 219 and her calf, No. 72. On 17th August, 1939, I was at Kakoma and saw the herd. It was in a pitiable condition. With only three or four exceptions, all

the animals looked fly-struck, and the herd appeared to be doomed to rapid extinction. They were being bitten daily, though not to the extent of being worried by tsetse, and grazing, though plentiful, was of poor quality. However, by the end of 1939, with the coming of spring grass, the survivors picked

TABLE III.

Month.	Animals positive for trypanosomes.
1938	
October...	Nos. 4, 9, 18, 19, 25, 35, 40, 61, 65, 67, 70, 226
November	Nos. 3, 5, 8, 9, 19, 25, 35, 61, 70, 71 (Calf of No. 226 born 25.10.38), 219
December	As November
1939	
January...	Nos. 3, 5, 8, 9, 18, 19, 25, 35, 65, 68, 70, 71, 219
February	Nos. 3, 5, 8, 9, 18, 40, 65, 68, 69, 219
March ...	Nos. 3, 5, 9, 65, 68, 70, 72 (Calf of No. 219; born 4.11.38), 219
April ...	Nos. 3, 5, 9, 65, 68, 71, 72, 219

up again, and on 31st December, 1939, the herd still consisted of Oxen Nos. 8, 19, 44; Heifers Nos. 3 and 18, and "Calves" Nos. 65, 66, 67, 68, 69, 70 and 71, and it was stated to be looking much better than when I saw it four months earlier.

The next time I inspected the herd was when I was last at Kakoma, on 9th August, 1940. Deaths since the beginning of the year had been "Calf" No. 67 in June, "Calf" No. 70 in July, and Ox No. 44 in August, shortly before my arrival. The composition of the herd, as detailed in Table IV, should be compared with that given in Table I.

TABLE IV.

COMPOSITION OF EXPERIMENTAL HERD ON 9TH AUGUST, 1940.

Animal's No. and description.	Main points of history.	Condition.	Blood-smear.
3 Black cow ... ..	Vaccinated 1934	Fair	N.T.S.
8 Black ox ... ..	M.I.D. 1934	"	"
18 Black and white cow...	Control	Poor	"
19 Black and dun ox ...	M.I.D. 1934	"	T.c. +
65 Brown and white heifer	Born of No. 89, 20.5.37	Fair	N.T.S.
66 Brown and white heifer	Born of No. 226, 19.9.37	Poor	"
69 Black and white bull...	Born of No. 219, October, 1937	Good	"
71 Black male yearling ...	Born of No. 226, 25.10.38	Fair	T.c. +

I picked out No. 19 as the worst of a poor lot, and destroyed him for postmortem examination. Before death he was in weak condition, showed cachectic alopecia, and oedema of hind legs and of eyelids. Visible mucous membranes were pale. Postmortem: Anaemia. General cachexia with local subcutaneous oedemas. Oedema of lymphatic glands, and prominence of haemolymph glands, particularly in pharyngeal and mediastinal regions. Some *Tumor splenis*, due chiefly to enlargement of Malpighian corpuscles. Areas of hepatic parenchymatous degeneration, and general thickening of liver capsule. Nephritis of undetermined nature, characterised by general pallor, and excess of cortex in relation to medulla. Petechiae below endocardium of left ventricle. Slight catarrhal gastro-enteritis. No ticks, worms nor other macroscopic parasites were noticed, and trypanosomes (*T. congolense*) were not numerous in any smears. *Cause of death*: Destroyed, as unlikely to recover from chronic trypanosomiasis.

The last time I saw the herd, and what may be considered the close of the experiment, was on 21st January, 1941. Owing to the war, the experimental station of Kakoma was closed, and the tiny remnant of experimental animals were moved to Shinyanga, in the Lake Province; the headquarters of the Tsetse Research Department. Here animals are bitten only occasionally by tsetse. All that were left were Nos. 3, 18, 65, 66, 68 and 71. All were in poor condition—a typically badly fly-struck little herd. (See Fig. 2, p. 167.)

Of the animals vaccinated or otherwise treated by SCHILLING in 1934, one only was alive. There was also one control. The other four were later calves of some of the mothers of the originally treated cattle. Born in light "fly," they were all infected naturally before they were a year old, and subsequently they were exposed to repeated superinfections. During their short lives there had been times when, in spite of trypanosomes, they had regained a measure of good health. Yet they could not retain this state of premunition. There is nothing about the whole experiment to furnish hope that we are within sight of any practical method of immunizing cattle against trypanosomiasis.

#### DISCUSSION.

It is natural that the subject of the practical immunization of domestic stock against African trypanosomiasis should have attracted much attention and provoked research. Some workers tackled the subject directly with large animals. Others experimented with small animals, and often with trypanosomes differing in species from those transmitted by tsetse, in the hope that success in the laboratory could be repeated in the field when the same technique was applied to larger animals and tsetse-transmitted trypanosomes. Hopes of success were sustained by observations regarding the ability of game animals to thrive in tsetse belts everywhere, and of certain races of domestic stock, notably on the west coast of Africa, to survive within belts of light fly. It seemed that between the almost complete insusceptibility to infection of some

wild animals, such as the baboon, and the low resistance of some domestic animals lay not only the specifically high resistance of wild ruminants, but the inherently high resistance of these local races of domestic stock, and that as these had somehow achieved immunity comparable with that of game, so, surely science could expedite the natural processes by which this high natural resistance had been brought about, and thereby make possible the artificial immunization of stock of low natural resistance.

In connection with most game, it was noticeable that the protection they enjoyed after exposure to infection was due to an *immunitas non sterilisans*; they remained in a state of labile infection, as SCHILLING calls it, or, to use the widely accepted French term, they achieved premunition rather than true immunity. It was expected, then, that any protection conferred on domestic stock would be of the same nature.

The earliest work on immunization was handicapped by imperfect differentiation of species of trypanosomes. Thus, prior to 1904, all forms of nagana were attributed to *T. brucei* alone, whereas actually this species is of low pathogenicity for cattle, and the natural resistance exhibited by an ox inoculated with it might easily be mistaken for a measure of immunity conferred by a previous infection. Those interested in this early work should consult the relevant chapters in KNUTH and DU TOIT's *Tropen-Krankheiten der Haustiere* (1921), which are excellently documented with references up to the end of 1918.

The imperfect success of treatment of animal trypanosomiasis with drugs, so that clinical recovery without complete suppression of parasites was often the best that could be hoped for, suggested that premunition thus achieved against one strain might be a basis on which a more general protection could be built. Thus BEVAN (1928) advocated deliberate infection, followed by carefully timed and regulated doses of an antimonial drug, as a means by which cattle would engender sufficient immunity to protect them even within fly-belts.

This avenue towards practical immunization was explored by PARKIN and HORNBY (1930), who concluded: (1) Bovines premunized against a particular strain of *T. congolense* are unaffected by reinoculation with the same strain; (2) Bovines premunized against one strain of *T. congolense* are practically as susceptible to inoculation with a different strain as bovines not so premunized; (3) Bovines, premunized against a particular strain and then inoculated with a different strain, do not show, as reflected by the red-cell count, such a progressive anaemia as bovines not so premunized, and thereby may have a better chance of attaining to a state of premunition against this second strain. Further work along the same lines by HORNBY and BAILEY (1932) indicated that it is usually an easy matter to premunize an ox against any one strain of trypanosome, but it is never certain that this premunition will not break down in the form of a relapse; and even when it is maintained, this premunition against one strain gives little or no protection against infection by other species of pathogenic trypanosome or even against infection by other strains of the same species. This laboratory finding is supported by many observations in the field. Twenty

years ago I concluded that in some districts a number of native cattle recover spontaneously from infection but that no great immunity results therefrom (HORNBY, 1921), and I still hold the same opinion. One can hardly hope to put animals in a more favourable condition to resist further infection than was the state of SCHILLING's herd when it was photographed in December, 1936. (Fig. 1.) All the older animals were premunized against one or more strains of one or two species of trypanosomes, and all were in good health. Yet, as we have seen, almost all were dead from trypanosomiasis 4 years later, and there are no surviving offspring from the eight heifers. On the west coast of Africa, where the breeds of cattle are somewhat different, a more reliable immunity is often achieved, but in East Africa every flock or herd encountered within a fly-belt is living precariously. The conditions relating to one such herd of twenty-one "immune" animals were described in some detail by HORNBY and BAILEY (1932)—the next year all the animals were dead; apparently from trypanosomiasis.

However, attempts to immunize cattle continue—as, of course, they should. SCHILLING believed that the best results are achieved when the animal gets its first inoculation while it is very young and, therefore, probably best able to resist. The belief that suckling animals offer more resistance to the effects of infection than older animals appears to be well founded. BEVAN (1936) states that this belief was held by such early African hunters and explorers as LIVINGSTONE, BAINES and SELOUS. There is also laboratory evidence in its favour, e.g., that furnished by HERRICK and CROSS (1936). It is the basis of the claim by VAN SACEGHEM (1938) that inoculation of *T. congolense* into a calf less than 1 month old leads to a real immunity in areas where trypanosomiasis due to this parasite is maintained merely mechanically by flies other than tsetse, that is, where only one or two strains are involved—a very different matter from the infections pertaining to a fly-belt. SCHILLING's method also takes advantage of youth, and particularly of youth aided by antibodies obtained *in utero* from a mother already premunized. Very young animals, preferably those suckling by premunized mothers, are inoculated with minimal infective doses of the three common species of pathogenic trypanosomes. Thereafter, they must be well looked after and must recover clinically before being exposed to the natural infections of fly-belts. SCHILLING believed that this method had been applied successfully to those animals which, in Table I, are shown as having received minimal infecting doses.

A less reliable though safer way of conferring protection was thought to be by the inoculation of dead trypanosomes, though one disadvantage was that *T. vivax* could not be used, since this species does not multiply freely in any small laboratory animal. This method is an old one and, in the laboratory, has given conflicting results (see PONSELLE, 1923), though some of the more recent work on the method, by KLIGLER and BERMAN (1935), seems to point clearly to the development of a specific enhanced resistance to infection following treatment with suspensions of dead trypanosomes. At any rate, SCHILLING

thought he had been successful in immunizing by this method the young cattle shown, in Table I, as treated by vaccination.

Attempts to immunize cattle by inoculation of living trypanosomes attenuated by normal passage through other host species were made as long ago as 1897 (KOCH, 1901), but were soon abandoned. PONSELLE (1923) claimed some success in the laboratory from the inoculation of cultures of trypanosomes, and a very modern suggestion, by BIOCCA (1939), is that trypanosomes in chick embryo blood kept on ice might be used for immunization purposes. Nevertheless, in spite of these and many other kinds of counsel, the only known way of protecting East African stock while these are in fly-belts is by continual drug treatment. SCHILLING thought he had devised a method of immunization that after refinement would be of great practical value, but the subsequent history of animals which he thought were really immune has shown that his conclusions were premature and his high hopes were not justified.

### CONCLUSION.

No method of immunization which will enable East African domestic stock to thrive in tsetse-fly belts has yet been devised.

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## DOES INFANTILE BERIBERI OCCUR IN INFANTS WHO HAVE NEVER BEEN BREAST-FED? \*

BY

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In 1888 HIROTA observed in infants, who were nursed by beriberic women, a complex of symptoms which he called "infantile beri-beri."

Years later, ITO (1911) observed similar symptoms in infants whose mothers were apparently healthy, and he called this condition "mother's milk intoxication." However, after having noticed that some of the infants were nursed by wet-nurses, he changed this name to "breast milk intoxication." Later investigators (INABA 1917, TOYADA 1922) pointed out that the symptoms of both infantile beriberi and breast milk intoxication tend to disappear after administration of vitamin B<sub>1</sub>. They inferred that the two conditions were in fact identical. It was suggested further that the apparently healthy women who had nursed infants showing breast milk intoxication must have been in a state of occult beriberi.

In 1934 TAKAMATZU extracted from the milk of women, whom he considered to be B-avitaminotic, a material which he called methyl glyoxal-like substance. Later on this substance was found by S. SATO (1939) to consist chiefly of methyl glyoxal, which is an intermediate toxic product in the metabolism of carbohydrates. Intermediate products, which are collectively termed B.B.S. (bisulfite binding substances), were found in increased quantities, as result of incomplete oxidation of carbohydrates, in the milk of B-avitaminotic women (PLATT and LU 1936).

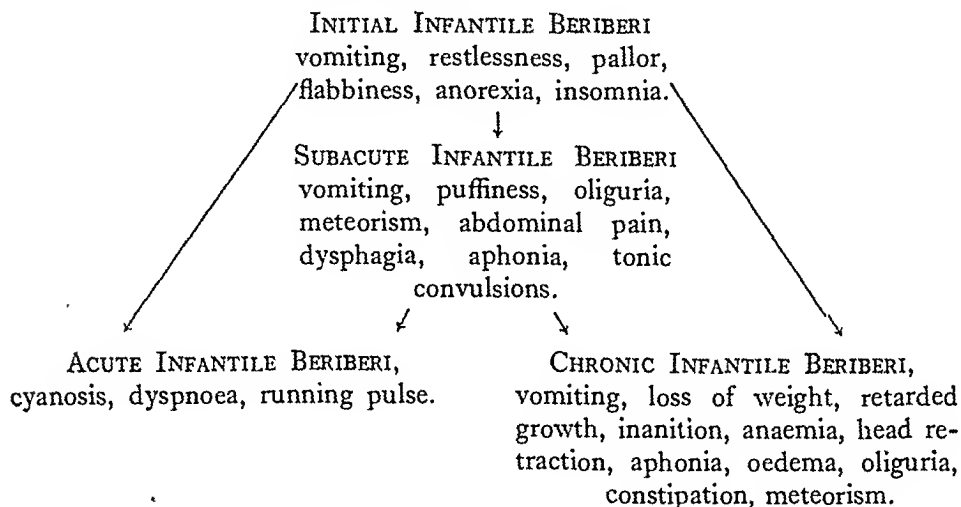
\* Read before the Hongkong Branch, British Medical Association, June 2nd, 1941.

† Vitamin B means vitamin B<sub>1</sub> throughout.



It seems that after ingestion of milk containing such intermediate products, the infant tries to get rid of them partly by excretion; but mostly by further oxidation to their end products. For this purpose vitamin B as co-carboxylase is absolutely essential. However, the milk of B-avitaminotic women is deficient in this vitamin and the infant's own vitamin reserve is therefore bound to be soon depleted. As a result the intermediate products accumulate, and at least one of them, namely methyl glyoxal, causes the above-mentioned infantile beriberi or breast milk intoxication.

According to the symptoms and the course infantile beriberi may be classified as follows (FEHILY, 1940):



Mixed forms are often observed such as initial or subacute infantile beriberi with the beginnings of cyanosis, dyspnoea and tachycardia.

The condition of the reflexes is variable. They may be exaggerated, normal, diminished or absent.

In all stages of infantile beriberi, probably due to B avitaminosis, the resistance against infections is greatly diminished especially against those of the respiratory tract. In fact bronchitis is usual and consequently this disease, as well as pyrexia caused by complications (mostly beginning bronchopneumonia), are regarded by some authors as symptoms of infantile beriberi. In uncomplicated cases the temperature is normal or subnormal.

Infantile beriberi commences usually in the first 100 days of life. Its appearance, however, may be retarded and it can appear at any time during the period of breast feeding. In Hongkong infants are often breast fed for a long period, sometimes even after completion of the second year.

The symptoms of infantile beriberi may be divided, according to acuteness, into symptoms due to intoxication and symptoms due to B avitaminosis. The symptoms of intoxication, such as vomiting, restlessness, abdominal pain,

cyanosis, dyspnoea, running pulse etc., prevail in the initial, subacute and acute forms of infantile beriberi, whereas chronic infantile beriberi is dominated by symptoms of B avitaminosis such as anorexia, retarded growth, loss of weight, constipation, etc.

As the last-mentioned symptoms are encountered also in infants who have never been breast-fed but who suffer from B hypo- or a-vitaminosis, these latter conditions may give rise to symptoms simulating infantile beriberi. These conditions in artificially fed infants are often diagnosed as malnutrition but, in contrast to chronic infantile beriberi, symptoms such as aphonia and head retraction, in the writer's experience, are not encountered in such B hypo- or a-vitaminotic states.

After cessation of breast-feeding the symptoms of intoxication promptly disappear, whereas those of avitaminosis persist for weeks or months, especially if the substitute foods used are deficient in vitamin B or its amount is insufficient to remedy the existing deficiency.

Regarding the occurrence of infantile beriberi, two schools of opinion prevail amongst the investigators on this disease. One group of authors which includes Japanese (HIROTA 1891, ITO 1911, INABA 1917, etc.), Philippine (GUERRERO 1904, GUERRERO and QUINTOS 1910, ALBERT 1931, etc.) and Chinese (CHAN 1935, KAO 1936) is of the opinion that infantile beriberi occurs only in breast-fed infants, whereas the other group, including the workers in the Netherlands East Indies (DE LANGEN and LICHTENSTEIN 1936) and the Federated Malay States (HARIDAS 1937, WILLIAMS 1938), reports that this disease is encountered also in those who are artificially fed.

This divergence of opinion might be due firstly to the fact that chronic infantile beriberi of breast-fed infants and B hypo- or a-vitaminosis of those who have never been breast-fed have many common symptoms. Secondly, the term "breast-fed infant" is not used by all authors in the same sense.

The first group mentioned above would appear to use the term "breast-fed" infant so as to include all infants who have, for any period, however short, received breast-feeding. Confirmation of this statement could be seen in a case reported by GUERRERO and QUINTOS (1910) in their monograph entitled *Beriberi in Breast-fed Infants*. In this monograph they reported a case of a 5-months'-old infant, fed since birth on condensed milk only, but who died of acute infantile beriberi after being nursed twice by a neighbouring mother. Obviously this child was not a breast-fed infant, although it had been breast-fed on these two occasions.

The second group of authors, on the other hand, appears to limit the term "breast-fed infant" to infants who have been, up to the time in question, entirely breast-fed. Consequently, instead of a statement "infantile beriberi occurs in breast-fed infants only," the following would be more correct: "infantile beriberi occurs only in infants, who have been, at some time, breast-fed."

The writer, after having observed 350 cases of infantile beriberi, has not encountered amongst them a case where it was positively proved that the infant had not received human milk. In the initial, subacute and acute stage, the breast feeding has taken place immediately before, whereas in chronic infantile beriberi the breast feeding might have taken place weeks or months previously. In many cases of infantile beriberi the parents or guardians denied emphatically that the infants had ever been breast-fed; however, investigation revealed always that their statements were incorrect. Sometimes such misstatements were deliberate with the intent to receive a greater amount of free condensed milk; sometimes the mother did not consider it worth while to mention one or two nightly breast feeds; or the fact that the child was given breast as a "dummy" in addition to artificial feeding. Furthermore, in Hongkong, there are many cases of so-called adopted children and, of course, their previous history is not always known to their new parents.

At first sight it may seem incomprehensible that infants born of vitamin B-deficient women, and fed since birth on vitamin B-deficient food, other than human milk, should not show signs of beriberi. As mentioned already, vitamin B hypo- or a-avitaminosis are also encountered in infants who have never been breast-fed. This is proved by the fact that such infants, with symptoms of anorexia, loss of weight, retarded growth and constipation improve immediately after the administration of vitamin B. Logically, it would follow that such infants, if unmedicated, should develop, in due course, beriberi, not of the infantile, but of the adult type.

However, the strange fact is that beriberi of the adult type is never encountered in a completely (*i.e.*, since birth) artificially fed infant. At first the writer assumed that in such cases the non-occurrence of the adult type of beriberi could be attributed to the infantile vitamin reserve, which protects them, during the first 12 months of their life, against pronounced avitaminosis. However, taking into consideration the fact that the vitamin B reserve in adults is depleted after an approximate period of 2 months' vitamin-free diet, the above-mentioned assumption seems to be quite improbable. In addition, even if it be presumed that the infant's vitamin reserve would suffice for the first 12 months, then the deficiency ought to manifest itself in beriberi some time between the first and second years. Here, again, between these ages, the writer was unable to observe the adult type of beriberi. In fact, in the literature on beriberi, various authors stated that beriberi is uncommon between the weaning age and early adult life. No satisfactory explanations are offered to account for this absence, which is all the more remarkable because vitamin B is more essential for growth than for maintenance and because the calorie requirements of infants and children are relatively greater than those of adults. Furthermore, in the case of the infants and children up to 2 years of age the diet is restricted and consists mostly of carbohydrates.

The writer suggests that the explanation may lie in the above-mentioned fact, *i.e.*, that vitamin B is more essential for infants and children. When it is deficient or absent artificially fed infants never, and children rarely, survive the early stage of B avitaminosis.

In the case of infantile beriberi the position is entirely different. The infants suffering from infantile beriberi were usually fed, since birth, on human milk. This milk, although deficient in vitamin B, most probably has, as far as Hongkong is concerned, a sufficient amount of vitamin D. Vitamins A and C may or may not be deficient and six samples of milk from beriberic women examined for their protein and fat content gave results within normal limits. Consequently, infants subject to infantile beriberi may progress satisfactorily until the products of incomplete carbohydrate metabolism begin to appear in human milk. Thereafter the transition from the state of B hypovitaminosis into the states of avitaminosis and infantile beriberi occurs rapidly within a few days. Consequently the infants who begin to show manifest signs of infantile beriberi are still in a good physical condition and their appearance certainly bears out this statement. Although the transition from a normal condition into that of infantile beriberi occurs suddenly, the infants, even in such a short time, are subject to intercurrent infections. Many infants succumb to such infections even in the stages of B hypovitaminosis and, as already stated, the majority of infants with manifest signs of infantile beriberi are suffering from bronchitis, which often ends fatally as bronchopneumonia.

The position of infants who, since birth, have been entirely artificially fed, is totally different. The majority of such infants receive, in Hongkong, diluted sweetened condensed milk or rice flour, made from highly milled rice. The first-mentioned food is deficient in all the essential vitamins, as well as fat, whereas rice flour is poor in protein also. Consequently the infants are in a state of malnutrition before vitamin B deficiency commences to manifest itself. The transition from the stage of B hypovitaminosis into the stage of B avitaminosis occurs insidiously over a period of weeks or months. Consequently the chances of contracting the intercurrent diseases are enormous and the chances for survival nil. As a result the artificially fed infants, who are vitamin B deficient, succumb before their deficiency becomes manifest in beriberi of the adult type.

#### SUMMARY.

1. The writer is of the opinion that infantile beriberi does not occur in infants who have never been breast-fed.

2. In the case of initial, subacute and acute infantile beriberi the breast-feeding must have taken place immediately before the occurrence of symptoms; in the case of chronic infantile beriberi the breast-feeding might have taken place weeks or months previously.

3. The symptoms of infantile beriberi are divided into those due to intoxication and those due to B avitaminosis.

4. Initial, subacute and acute infantile beriberi are dominated by symptoms of intoxication, whereas chronic infantile beriberi is dominated by those of B avitaminosis.

5. After cessation of breast-feeding the symptoms of intoxication disappear promptly, whereas those of B avitaminosis may persist for weeks or months.

6. Chronic infantile beriberi is similar to conditions due to B hypo- or a-vitaminosis in infants who have never been breast-fed.

7. The writer is of the opinion that beriberi of the adult type is not encountered in artificially fed infants.

8. It is suggested that the explanation may lie in the fact that the artificially fed infants succumb to intercurrent diseases before their vitamin B deficiency becomes manifest in beriberi of the adult type.

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## OBSERVATIONS ON THE DIET OF TRINIDAD OILFIELD LABOURERS.\*

BY

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During the last decade labour disputes, strikes and riots, said to have arisen out of discontent due to an extremely low standard of living among the labouring classes, led to the setting up of boards, committees and commissions for the investigation of living conditions in the colony of Trinidad.

The Wages Advisory Board, in 1935, among other work, adopted the diet proposed by LASSALLE (1935) as being the minimum diet which would keep a man in full work in health. This diet, known locally as the "Lassalle diet," assumes that the man in question is in full health, leaving out the possibility of his suffering from one of the prevalent diseases (avitaminosis, ankylostomiasis, syphilis, gonorrhoea, malaria).

The Board also inquired into the articles of diet purchased by Trinidad workers, the list of which, together with the food-, calorie- and vitamin-values which I have added, is shown in Table II. A serious defect in this list is the fact that no attempt has been made to indicate which articles of diet are most often bought. Thus, sardines, tomatoes and beef are listed, but these are rare luxuries for the majority. Even when such an article as beef is purchased it is usually of the cheapest quality, containing for the most part bone and indigestible material.

### LASSALLE DIET.

				<i>Per Week.</i>		<i>Per Day.</i>
Meat or fish .. .. .	..	..	..	3½ lb.	=	½ lb.
Potatoes or vegetables	..	..	..	7 lb.	=	1 lb.
Bread or biscuit .. ..	..	..	..	8 lb.	=	1 lb. 2½ oz.
Sugar .. .. .	..	..	..	1½ lb.	=	3½ oz.
Butter or lard .. .. .	..	..	..	1½ oz.	=	2 oz.

The daily diet, as given above, may now be analysed (Table I).

\* Abstracted from M.D. thesis, University of Cambridge.

TABLE I.  
ANALYSIS OF LASSALLE (DAILY) DIET.

Article.	Cal- ories.	Protein. Gramme.	Fat. Gramme.	C'hyd. Gramme.	Minerals (mg.).			Vitamins.				
					Ca.	P.	Fe.	A	B <sub>1</sub>	B <sub>2</sub>	C	D
1. Meat or fish—8 oz.												
Beef (lean)	320	50	14	VL	22	418	48	VL	+	++	VL	VL
Beef (fat)	560	36	44	VL	22	110	48	VL	+	++	VL	VL
Fish (salt)	520	74	11	9	208	160	—	VL <sup>*</sup>	VL	VL	VL	VL
Average	467	53	23	3	84	229	32	VL	+	+	VL	VL
2. Potatoes or vegetables—16 oz.												
Potato (sweet)	544	5	2	64	160	160	10	VL	+	++	++	—
Tannia	576	10	0	102	—	—	—	—	—	—	—	—
Yam	544	6	0	122	160	0	5	+	+	+	VL	—
Cassava	640	3	2	160	160	160	5	VL	VL	VL	VL	VL
Average	576	6	1	112	160	107	7	VL	+	+	VL	VL
3. Bread or biscuit—18½ oz.												
Bread	1,220	46	0	344	93	416	5	VL	VL	VL	VL	VL
Biscuit	1,220	46	0	344	93	416	5	VL	VL	VL	VL	VL
Average	1,220	46	0	344	93	416	5	VL	VL	VL	VL	VL
4. Sugar—3½ oz.												
Sugar	385	0	0	600	0	0	0	O	O	O	O	O
5. Butter or lard—2 oz.												
Butter	436	1	48	VL	—	—	—	+++	O	O	O	++
Lard	514	—	57	—	—	—	—	O-+	O	O	O	O
Average	475	0.5	52	VL	—	—	—	+	O	O	O	+

The sum of the averages above gives the following figures as the daily ration:—

Calories.	Protein. Gramme.	Fat. Gramme.	C'hyd. Gramme.	Minerals (mg.).			Vitamins.				
				Ca	P	Fe	A	B <sub>1</sub>	B <sub>2</sub>	C	D
3,117	105	76	1,060	237	752	44	VL	VL-+	VL	VL	VL

+++ = a very good source of vitamin    ++ = a good source of vitamin.    + = a moderate source of vitamin.  
VL = very little vitamin,    O = no vitamin,    — = data inadequate.

Comparison of the Lassalle diet with those designed by STIEBELING (? date) in U.S.A. and CLEMENTS (1940) for tropical Australia is of interest, the more so when it is remembered that CLEMENTS' diet (the more liberal of the two) is intended for white Australians doing a moderate amount of work: the West Indian labourer is expected to perform very heavy work. The diets are shown side by side in Table III.

CLEMENTS' scale of foodstuffs, covering a week's dietary for an Australian worker in the tropics, gives a far more varied and interesting diet than those evolved either by LASSALLE or the Wages Advisory Board, and is shown in Table IV. It serves as an indication of what future dieticians in the West Indies should allow.

From Table III it will be seen that the Lassalle diet compares very unfavourably with those of other authorities. Unfortunately, this diet does not convey a true picture of the actual state of affairs; the actual diet of the average Trinidad oilfield labourer is far less extensive than even that recommended by LASSALLE.

Racial differences must be taken into consideration whenever the question of diet arises, although the Wages Advisory Board in its report, cited above, states: "... The difference is not great, and we have considered it desirable to deal with classifications of labour rather than with the often rather obscure racial differences between workmen performing the same class of work."

The negro, when possible, is a hearty trencherman, aiming at a sensation of complete repletion at the end of each repast, but his diet is far more limited than the diet of LASSALLE would suggest. The figure of  $3\frac{1}{2}$  lb. a week for meat or fish is far higher than usually obtains. 1 lb. of salt fish (cod or ling, imported from Newfoundland) per week is nearer the correct figure; and this article, as shown in Table II, contains very little of any of the vitamins. Meat is an occasional luxury, consumed less often than once weekly, and even then purchased as "pieces," "scrag ends" or bones: these contain much useless and indigestible matter.

Since the heavy manual work of the negro demands an abundance of calories these are made up with the cheaper carbohydrates and vegetables ("ground provisions"). A staple for his midday meal is the "Johnny cake" or "bake," made from 1 lb. of white flour and cooked in edible (coconut) oil. Since the "Johnny cake" contains a large amount of energy-producing carbohydrate and produces a sensation of repletion in the stomach, it is a very popular article of diet, but, owing to its coating of oil, it is extremely indigestible and produces digestive disorders in later life. The "Johnny cake" contains small and inadequate amounts of vitamins A and B<sub>2</sub>.

A second source of energy is white (polished) rice, which may be consumed in amounts exceeding 1 lb. per day. This foodstuff contains small amounts of vitamins B<sub>2</sub> and C and none of the others.

"Ground provisions" (vegetables)—sweet potatoes, dasheen, canna, tannias, yams, cush-cush—are another source of carbohydrate which also contain very small amounts of vitamins A, B<sub>1</sub>, B<sub>2</sub> and C.



TABLE II.

No.	Article of Diet.	Mois- ture (per cent.)	Protein (gramme per oz.)	Fat (gramme per oz.)	Carbo- hydrate (per cent.)	Cal- ories per oz.)	Cal- cium per cent.)	Phos- phorus per cent.)	Iron (mg. per cent.)	Vitamins					No.
										A	B1	B2	C	D	
1	Beef (lean) ...	71	6.2	1.7	Very little	40	0.01	0.19	2.0	VL	+	++	O-+	VL	1
2	Beef (fat) ...	63	4.5	5.5	Very little	70	0.01	0.19	2.0	VL	+	++	O-+	VL	2
3	Beef (salt) ...	?	?	?	Very little	?	?	?	?	?	?	?	?	?	3
4	Beef (corned) ...	?	?	?	Very little	?	?	?	?	?	?	?	?	?	4
5	Pork (fresh) ...	75	5.3	1.4	Very little	34	0.02	0.20	?	VL	++	++	O-+	?	5
6	Pork (salt) ...	?	?	?	Very little	?	?	?	?	?	?	?	?	?	6
7	Pigs' heads ...	?	?	?	Very little	?	?	?	?	+	O	O	O	O	7
8	Pigs' snouts ...	?	?	?	Very little	?	?	?	?	?	?	?	?	?	8
9	Pigs' tails ...	?	?	?	Very little	?	?	?	?	?	?	?	?	?	9
10	Ribs (beef). As with lean beef, but a large proportion of useless bone is included in the purchase.	?	?	?	?	?	?	?	?	?	?	?	?	?	10
11	Fish (salt cod) ...	37	9.3	1.4	4.0	65	1.00	0.70	6.0	VL	VL	VL	VL	VL	11
12	Fish (salt, other) ...	?	?	?	?	?	?	?	?	?	?	?	?	?	12
13	Salmon (salted) ...	48	8.0	8.0	0.5	92	0.20	0.16	VL	+	+	VL	?	?	13
14	Salmon (canned) ...	?	?	?	?	?	?	?	?	?	?	?	?	?	14
15	Sardines (canned) ...	?	?	?	?	?	?	?	?	?	?	?	?	?	15
16	Butter ...	14	0.3	24.0	Very little	218	?	?	0	++	O	O	O	++	16
17	Lard substitute ...	?	?	?	?	?	?	?	?	O-+	O	O	O	?	17
18	Oleomargarine ...	?	?	?	?	?	?	?	?	+	+	+	+	+	18
19	Milk (fresh) ...	87	0.9	1.1	5.0	19	0.12	0.09	VL	++	++	++	VL	++	19
20	Milk (canned) ...	29	2.3	2.3	54.0	?	?	?	?	?	?	?	?	?	20
21	Bread (white) ...	40	2.6	0.6	48.0	70	0.04	0.25	2.0	VL	++	+	VL	++	21
22	Biscuits ...	?	?	?	?	?	?	?	?	?	?	?	?	?	22
23	Corn meal ...	13	2.6	0.8	70.0	97	0.12	0.34	4.0	+	+	VL	VL	O-+	23
24	Shelled corn ...	13	2.6	0.8	70.0	97	0.12	0.34	4.0	+	+	VL	VL	O-+	24
25	Corn on cob ...	79	1.1	0.1	15.0	23	0.01	0.10	8.0	+	?	VL	VL	?	25
26	Flour (wheat) ...	14	2.8	0.4	73.0	98	0.02	0.10	1.0	VL	++	VL	VL	O	26
27	Oats ...	10	3.9	2.0	64.0	106	0.05	0.42	4.0	VL	++	?	VL	O	27
28	Rice (brown) ...	13	2.3	0.3	76.0	99	0.02	0.20	3.0	VL	++	VL	VL	O	28
29	Rice (polished) ...	13	2.0	0.1	78.0	97	0.01	0.10	2.0	O	O	VL	VL	?	29
30	Beans (red) ...	12	5.4	0.3	59.0	92	?	?	?	VL	VL	VL	VL	O	30
31	Beans (white) ...	?	?	?	?	?	?	?	?	VL	VL	VL	VL	O	31
32	Dholl (yellow) ...	13	6.2	0.6	59.0	97	0.14	0.25	9.0	++	++	++	VL	?	32
33	Peas (blackeye) ...	11	7.0	0.2	55.0	90	0.20	0.37	9.0	++	++	++	VL	?	33
34	Peas (pigcon) ...	12	6.2	0.2	56.0	90	0.25	0.40	7.0	++	++	?	VL	?	34

35	Peas (split) ...	1.4	5.6	0.2	58.0	90	0.08	0.30	6.0	+	+	+	VL	?	35
36	Cassava ...	62	0.2	0.1	35.0	40	0.04	0.04	1.0	VL	VL	+	VL	?	36
37	Potato (sweet) ...	68	0.3	0.1	28.0	34	0.04	0.04	2.0	VL	+	+	+	?	37
38	Tannia ...	71	0.6	0.0	25.0	36	?	?	?	?	?	?	VL	?	38
39	Yam ...	69	0.4	0.0	28.0	34	0.06	0.02	1.0	+	+	+	+	+	39
40	Breadfruit ...	74	0.4	0.1	22.0	27	0.04	0.04	2.0	+	+	+	?	?	40
41	Plantain ...	84	0.3	0.0	14.0	17	0.02	0.04	2.0	+	+	+	+	+	41
42	Pumpkin ...	91	0.3	0.3	0.0	8	0.02	0.02	2.0	+	+	+	+	?	42
43	Coconut ...	31	1.4	1.4	15.0	137	0.02	0.12	1.0	VL	VL	+	VL	?	43
44	Garlic ...	66	1.4	1.4	27.0	?	0.02	0.30	1.0	VL	?	?	+	?	44
45	Pepper (black) ...	14	3.1	2.6	48.0	?	0.46	0.19	17.0	?	?	?	+	?	45
46	Carrot ...	?	?	?	?	?	?	?	?	+	+	+	+	?	46
47	Turnip ...	?	?	?	?	?	?	?	?	O-+	+	+	+	?	47
48	Tomato ...	94	0.4	0.0	4.0	7	0.01	0.01	1.0	+	+	+	+	?	48
49	Ochro ...	90	0.6	0.0	7.0	10	0.09	0.06	2.0	+	+	+	+	?	49
50	Chives ...	66	1.4	0.0	27.0	?	0.02	0.30	1.0	VL	?	?	+	?	50
51	Cush-cush ...	?	?	?	?	?	?	?	?	?	?	?	?	?	51
52	"Herbs, greens" ...	?	?	?	?	?	?	?	?	?	?	?	?	?	52
53	Onion ...	90	0.3	Very little	9.0	11	0.15	0.05	VL	VL	+	+	+	?	53
54	Cocon ...	?	?	?	?	?	?	?	?	O	?	?	?	O	54
55	Eddoes ...	?	?	?	?	?	?	?	?	?	?	?	?	?	55
56	Molasses ...	?	?	?	?	?	?	?	?	O	+	+	?	?	56
57	Coconut oil ...	?	?	?	?	?	?	?	?	O-+	O	O	O	?	57
58	Olive oil ...	?	?	?	?	?	?	?	?	O-+	O	O	O	O-+	58
59	Cane sugar ...	?	?	?	?	?	?	?	?	O	O	O	O	?	59

## Addendum : Articles of diet not on the Board's list, but which have a place in the Trinidadian dietary.

1	Barley ...	11	3.4	0.4	70.0	97	0.04	0.35	4.0	VL	+	+	VL	VL	1
2	Water melon ...	95	0.1	0.1	4.0	5	0.01	0.01	VL	?	?	?	?	?	2
3	Banana ...	61	1.3	0.2	35.0	41	0.01	0.04	VL	VL	+	+	?	VL	3
4	Mango ...	84	1.0	0.1	12.0	15	0.01	0.02	VL	+	?	?	+	?	4
5	Pawpaw ...	89	2.5	0.1	8.0	12	0.01	0.01	1.0	+	?	?	?	?	5
6	Sugar-cane ...	87	0.1	1.0	12.0	15	?	?	?	?	?	?	?	?	6
7	Guava ...	81	1.0	0.3	10.0	13	0.02	0.03	1.0	+	?	?	+	?	7
8	Lime ...	88	1.0	0.1	9.0	11	0.07	0.02	VL	O	?	?	+	?	8
9	Orange ...	88	0.8	0.2	10.0	12	0.04	0.03	1.0	+	+	+	+	?	9
10	Canna ...	13	0.06	0.1	86.0	99	0.01	0.02	?	O	O	O	O	?	10

++++ = a very good source of vitamin.

++ = a good source of vitamin.

+ = a moderate source of vitamin.

VL = very little.

O = vitamin absent. ? = data inadequate.

TABLE III.

COMPARISON OF THE STIEBELING, CLEMENTS AND LASSALLE DIETS.

	STIEBELING.	CLEMENTS.	LASSALLE.
Calories .. .. .	3,600	3,800	3,117
Protein (grammes) ..	100	120	105
Fat (grammes) .. ..	—	115	76
Carbohydrates (grammes)	—	470	1,060
Calcium (milligrammes) ..	680	1,120	237
Phosphorus (milligrammes)	1,320	1,720	752
Iron (milligrammes) ..	15	21	44
Vitamin A (International Units)	4,000	6,650	Very little
Vitamin B (International Units)	350-450	450	—
Vitamin B <sub>1</sub> .. .. .	?	?	Very little— Moderate
Vitamin B <sub>2</sub> .. .. .	?	?	Very little
Vitamin C (milligrammes)	50	50	Very little
Vitamin D .. .. .	?	?	Very little

Some second class protein is supplied in the pigeon pea (*Cajanus indicus*) which also contains fair quantities of vitamins A, B<sub>1</sub> and B<sub>2</sub>. Some of these vitamins, especially A and B<sub>1</sub> are destroyed by the creole method of cooking.

Even when comparatively affluent, the East Indian rarely enjoys the luxury or variety attained by the negro. Apart from religious prohibitions, he is seldom or never in a position to buy meat. An ineradicable instinct for hoarding prompts him to reduce his food intake in order to save a few cents, and his diet is practically an all-rice one, cut down to a minimum—in some instances to as little as 8 oz. a day. Flavouring, in the form of leaves, “bush” or peppers, may supply a few vitamins (especially A and C), but the vitamin B complex is grossly deficient.

The deficient diet of the worker is also found in the pregnant and lactating woman and it is the lack of vitamins A and D during pregnancy and lactation which is probably responsible, in part, for the dental disturbances so prevalent in Trinidad.

Many children are either never breast-fed at all or are weaned at a very early age to enable the mothers to go out to work. Artificial feeding is often of a very crude and irregular type and may be entrusted to senile and unreliable relations. Some infants receive a diet composed almost exclusively of barley water, which has been shown to have an anticalcifying effect on the developing teeth, leading to flaws in the dentine. Other infants are fed on cheap and poor patent milk-foods, low in proteins and vitamins.

The growing child is subjected to the same deficient diet as his parents.

TABLE IV.

CLEMENTS' SCALE.

(Scale for a balanced and not unduly monotonous diet. The quantities shown represent the weekly allowance for one man and more than the minimum essential food constituent has been provided on the assumption that some of the men will not eat the full quantities of those foodstuffs containing essential constituents. It is assumed that those foodstuffs in *italic* will be eaten in full.)

Whole powdered milk ..	12 oz.	Prunes .. ..	2 oz.
Skimmed powdered milk	4 oz.	Mixed preserved meats ..	5½ lb.
<i>Cheese</i> .. ..	5½ oz.	Bacon .. ..	7 oz.
<i>Dried haricot beans</i> ..	6 oz.	Bread (white) .. ..	6 lb.
Dried split peas .. ..	1 oz.	Cocoa .. ..	1 oz.
Brown rice .. ..	2 oz.	Canned vegetables (peas,	
Potatoes .. ..	5½ lb.	string beans, carrots,	
Sugar .. ..	14 oz.	cauliflower) .. ..	2 lb.
Jam, syrup or treacle ..	14 oz.	Canned fruits (e.g., pine-	
Butter (canned) .. ..	14 oz.	apple) .. ..	12 oz.
<i>Oatmeal or wholemeal</i> ..	12 oz.	Tea, salt, etc.	
<i>Canned tomatoes</i> .. ..	3 lb.	<i>Vitamin B preparation</i> , 1 tablespoon-	
<i>Dried apricots</i> .. ..	4 oz.	ful daily.	

Calcium and phosphorus deficiency are probably comingled with a general vitamin lack and the deficiency of these two minerals is evidenced by two common conditions—pica (earth-eating) and “worm fits.”

Earth-eating is very common among small children in Trinidad and is popularly attributed to ankylostomiasis, idiocy and other diseases. That it is not necessarily due to hookworm disease is shown by the fact that it frequently occurs in children whose stools are free of ova or in children already cured of the disease. In my experience, earth-eating is a far commoner condition than mental deficiency and often occurs in children with average intelligence. Where pica occurs with idiocy other stigmata are usually present: I recall two such cases—one in a white creole suffering from mongolism and the other in an East Indian microcephalic. In both, other perversions, including coprophagia, were present.

NICHOLLS (1938) states that pica is almost certainly a sign of mineral deficiency, and that where a supply of common salt is adequate the deficiency is of calcium or phosphorus or both. It is likely that many of the “worm fits” of Trinidad are due to this cause.

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TABLE V.

BOTANICAL NAMES OF TRINIDAD FOOD PLANTS, COMPILED FROM MACMILLAN (1935)  
AND NICHOLLS (1938).

1.	Banana .. ..	<i>Musa sapientum</i> (Scitamineae).
2.	Breadfruit .. ..	<i>Artocarpus incisa</i> (Urticaceae).
3.	Canna .. ..	<i>Maranta arundinacea</i> (Scitamineae).
4.	Cassava .. ..	<i>Manihot utilissima</i> (Euphorbiaceae).
5.	Chives .. ..	<i>Allium schoenoprasum</i> (Liliaceae).
6.	Corn .. ..	<i>Zea mays</i> (Gramineae).
7.	Cush-cush .. ..	<i>Dioscorea trifida</i> (Dioscoraceae).
8.	Eddo (Dasheen) ..	<i>Colocasia</i> var. (Aroideae).
9.	Guava .. ..	<i>Psidium guyava</i> (Myrtaceae).
10.	Mango .. ..	<i>Mangifera indica</i> (Anacardiaceae).
11.	Ochro .. ..	<i>Hibiscus esculentus</i> (Malvaceae).
12.	Pawpaw .. ..	<i>Carica papaya</i> (Caricaceae).
13.	Pea, Blackeye .. ..	<i>Vigna catieng</i> .
14.	Pea, Pigeon .. ..	<i>Cajanus indicus</i> .
15.	Pepper, Black .. ..	<i>Pimenta officinalis</i> (Myrtaceae).
16.	Pepper, Red .. ..	<i>Capsicum</i> spp. (Solanaceae).
17.	Plantain .. ..	<i>Musa paradisiaca</i> (Scitamineae).
18.	Potato, Sweet .. ..	<i>Ipomoea batatas</i> (Convolvulaceae).
19.	Tannia .. ..	<i>Colocasia antiquorum</i> ( <i>C. esculenta</i> ) (Aroideae).
20.	Water melon .. ..	<i>Citrullus vulgaris</i> (Cucurbitaceae).
21.	Yam .. ..	<i>Dioscorea cayensis</i> (Dioscoraceae).

## SUMMARY.

1. An account is given of the diet of Trinidad oilfield workers.
2. A standard diet, considered suitable for Trinidad labourers, is contrasted with standard diets in U.S.A. and tropical Australia.
3. Articles of food in common use in Trinidad are listed together with their calorie, mineral and vitamin content.
4. Common food-plants of Trinidad are listed (Table V).

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## INFLUENCE OF MANDELIC ACID ON THE COURSE OF TYPHOID FEVER.

BY

J. KLEEBOERG, M.D.,

*From the Medical Department A. Hadassah-Rothschild University Hospital, Jerusalem.*

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The good results obtained by mandelic acid therapy in cases of *Bacillus coli* pyelitis are based on two facts; this bacillus grows with difficulty in an acid medium and mandelic acid has proved to be a powerful bacteriostatic agent (ROSENHEIM). Since the typhoid bacilli have very similar characters it was thought worthwhile to try this treatment in cases of typhoid fever. CLARK and HELMHOLTZ (1931) and HELMHOLTZ and OSTERBERG (1934) have recorded the results of this therapy in pyelitis, while KOLMER (1940) has given a complete review of its use. Several authors have pointed out the contra-indications. PLICHET (1937) found that renal insufficiency with high blood urea, as well as high temperature, prohibit the use of mandelic acid. On the other hand, nausea, slight haematuria and occasional casts in the urine do not mean that this treatment should be stopped. Care in the continuation of the use of mandelic acid is also needed in cases of vertigo, headache and loss of appetite, though an immediate cessation is not indicated (ROSENHEIM). Some authors investigated the influence of mandelic acid and its salts on the mucous membrane of the stomach. It seems from observations made by GORDONOFF (1939) in the Bürgis Institute that free mandelic acid can cause gastritis, but whether these findings from experiments on rabbits can be applied to human pathology still awaits proof. In any case its possibility must receive consideration especially from the point of view of the problem under consideration.

We started with an obvious experiment: a young female patient with typhoid fever of medium severity developed a violent specific cystitis, the urine being full of typhoid bacilli. Here we had every right to use the mandelic acid

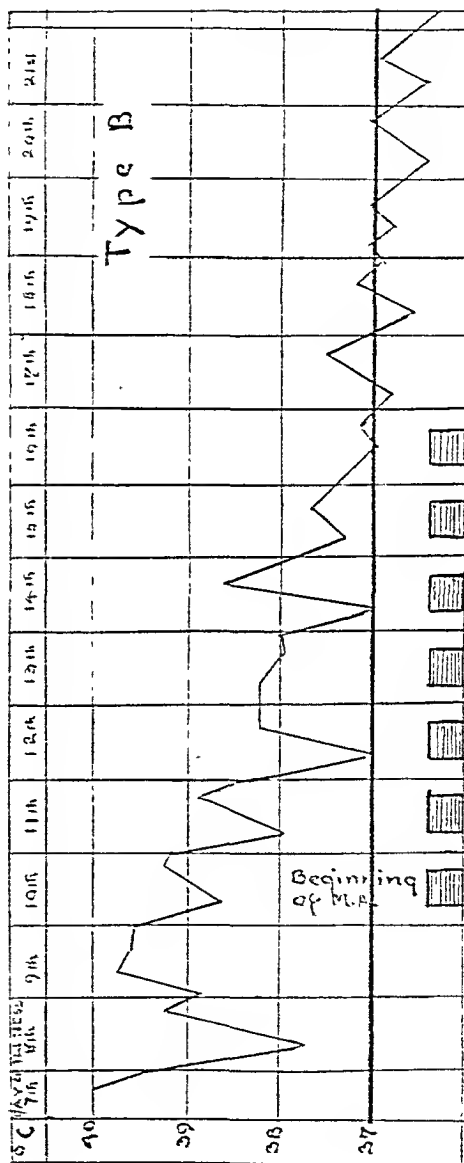
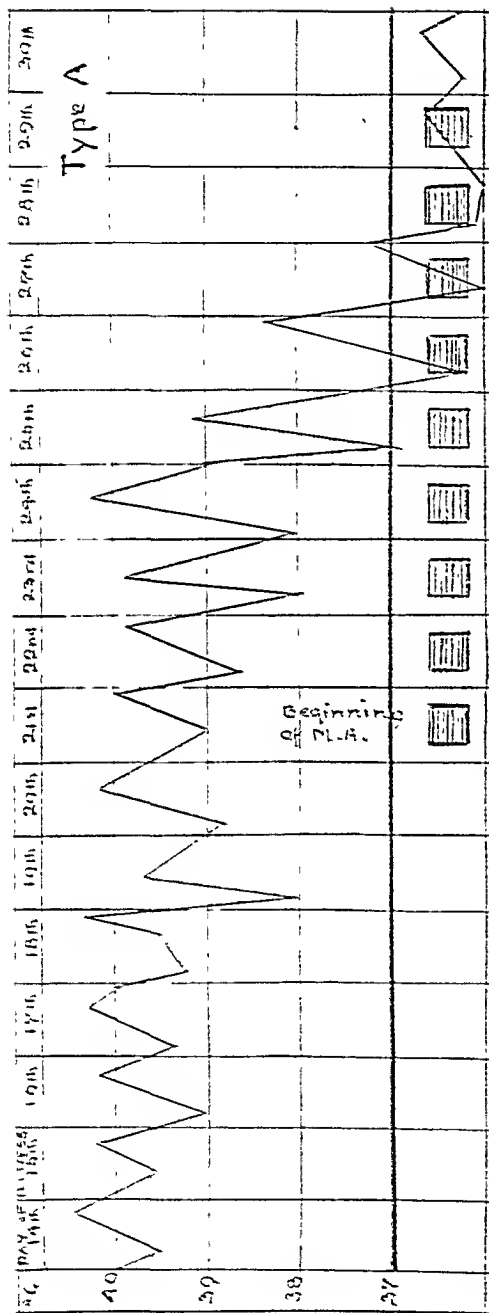


therapy. The temperature fell, and though the patient felt better, the urine cultures remained positive. As the medicament had been so well tolerated and the general state of the patient during the time of treatment had been so good, we felt justified in trying mandelic acid in other cases. First of all the patients were allowed to remain some days without any treatment (except perhaps a sleeping draught or some assistance against circulatory trouble) in order to observe the character of the disease. Cases with pneumonia or with early collapse or haemorrhage were excluded; the course of typhoid cases with such severe complications may be so unexpected and difficult to control, that they do not permit of reliable judgment as to the success or failure of any new treatment. Also mild cases and those in which the temperature seemed to drop, were excluded. The mandelic acid treatment was therefore reserved for patients who had already been ill for a long time at home, who still showed an elevated temperature and in whom no sign of spontaneous recovery was to be expected. Having examined the blood pressure, the blood urea and the urine and keeping the patients under constant control in regard to these facts, the treatment was commenced.

The mandelic acid and the acidifying salts were administered to the patient in the same way as in cases of pyelitis for 7 or 9 days. The intake of fluid was not restricted, for otherwise the patient with a high temperature would have suffered from thirst. The usual diet given in typhoid cases (KLEEGER, SEEFF) seemed to us permissible provided the amount of milk or fruit juice did not change the acidity of urine which was of course constantly controlled to prevent it reaching a higher level than pH 5.

Two kinds of results were obtained in those cases which responded favourably to the treatment: Type A which showed no fall in the temperature in spite of several days' administration of the drug, till suddenly and rapidly it dropped and remained normal; and Type B in which almost from the first day, but certainly from the second day, the temperature curve gradually and constantly dropped so that after 7 to 9 days it reached and remained at normal.

One strange case may be mentioned because of its unusual course: A boy of 13 came into the hospital with severe typhoid toxic symptoms, drowsiness with a temperature of  $40^{\circ}\text{C}.$ ; at home the temperature had been  $40.2^{\circ}\text{C}.$  After several days' observation we started with the mandelic acid treatment. This was without effect on the temperature or general symptoms but the patient was less drowsy and started to eat. Though his weight was low, being a thin and small boy, he received the full adult dose. On the 9th day of treatment a rigor was observed and the temperature dropped from  $41^{\circ}$  to  $35^{\circ}\text{C}.$  without collapse. Following this the temperature remained low and recovery began. Six days later the temperature again rose but without any general symptoms. A relapse was feared, but after 3 days, during which the temperature remained elevated ( $38.3^{\circ}\text{C}.$ ) the patient was given a second course of mandelic acid. Within 3 days the temperature fell to  $37.5^{\circ}\text{C}.$ , and after another 3 days was down to normal. The boy recovered.





## DISCUSSION.

Discussing our observations we will start with those which are negative and uncertain. First of all: was any harm done to the body or organs? We can definitely state that there was none. All those who were free from kidney trouble or gastric ulcers and who received the treatment tolerated the drug for 7, 8, 9 or 10 days and even a second course after 10 or 14 days' interval without any sign of trouble, as is already well known to be the case in the treatment of pyelitis.

Mandelic acid is well tolerated even during very high fever. Mild febrile albuminuria is no contra-indication. It must be admitted that some cases (four) did not respond, though we found no special reasons for this. Perhaps the doses of mandelic acid prescribed for adults four times a day (the usual routine for cases of pyelitis) is not sufficient for the severe typhoid septicaemia. As the case of the 13-year-old boy shows, larger doses can be given, so that further trial doses given five times a day could be made. One of the four cases received mandelic acid treatment for 8 days, and after an interval of 5 days a second course of 4 days' treatment. Although the temperature had remained above 39° C. it dropped when the second course was commenced.

The question, whether relapses can be prevented, either by the method of treatment described or by another cannot be judged from our small figures. More cases should be treated by giving after 8 or 10 days' interval a second course of mandelic acid during the fever-free period, in order to find out the effect of preventing the typhoid relapse. We are trying to find answers to all these questions.

It has to be noted that in none of the treated cases did the typhoid bacilli disappear from the stool or urine more quickly than in untreated cases.

On the other hand, we can say that a number of favourable features could be observed. Most of the patients receiving mandelic acid became less toxic, less drowsy, started to breathe more easily, were more alert and also began to eat better. In ten cases out of fourteen the temperature dropped steadily, the patient continuing to recover, mostly without interruption. Comparing the average duration of the same number of treated and untreated cases (selected as far as possible as being of the same type and severity) the length of illness of treated patients was 26 days and of untreated ones 31 days; a small but definite advantage.

Two patients (female), in addition to typhoid, suffered from an acute (specific) inflammation of the gall-bladder with definite, though not violent, symptoms. One recovered from both diseases within 7 or 8 days of mandelic acid treatment. The second patient who previously had suffered from attacks of colitis and cholecystitis did not react favourably to the mandelic acid treatment as regards the typhoid fever, but the gall-bladder pain and the sensitiveness of that region definitely diminished.

These two cases are mentioned merely to show that such a complication as a cholecystitis is not a contra-indication to mandelic acid treatment. Here, too, more cases should be observed for accurate judgment. One severe case, after the treatment, which was without influence on the severity of the disease, developed multiple perforation and died after operation. Two cases of psychosis, one with delirium, were also uninfluenced by the treatment.

### CONCLUSIONS.

The course of typhoid fever is so variable that only large numbers of cases can establish the value of any new treatment. Bearing this in mind it is nevertheless possible to draw the following conclusions:—

(1) As there is a biological relationship between *B. coli* and *B. typhosus* mandelic acid therapy effective against *B. coli* infection may also be effective against typhoid infection.

(2) The drug in none of our fourteen cases has shown any ill-effect.

(3) In ten of the fourteen cases the fever was distinctly influenced by the mandelic acid therapy, either immediately by lysis or some days later almost by crisis. After the fall of temperature the patient recovered. In all cases the general condition, breathing, mental state, appetite became better. The duration of the disease seems to have been shortened.

(4) Severe cases with complications were not influenced. The bacilli did not disappear more quickly from the urine or stools than in untreated cases.

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## THE NOMENCLATURE OF THE PACIFIC FILARIA

BY

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MANSON-BAHR (1941) raises again the question of the specific identity of the filaria found in the Pacific Islands and argues that it should have specific rank. As long ago as 1912 SIR PATRICK MANSON and myself agreed that specimens of filarial embryos sent by DR. BAHR from Fiji were identical with those of *Filaria bancrofti* (now known as *Wuchereria bancrofti*); and when adults were brought home later and examined by Professor LEIPER a similar identity between them and *W. bancrofti* adults was established. Morphologically, then, the two are the same both in their adult and embryonic forms, so it is not permissible to make them into two separate species. To get over this difficulty MANSON-BAHR brings in some biological points in which they differ and would, as he says, "in the biological sense," create a new species for the Pacific form. I am afraid systematists will not allow this, as species are not clinical entities or biological strains, but are based on morphology, and if this rule were changed all our existing classifications would fall to pieces and collapse.

The case of *Wuchereria malayi* is also somewhat difficult, but though there are at most only very slight differences between the adults, yet the embryos can be morphologically separated. BRUG, after his discovery in 1927, sent me specimens (embryos), and I agreed that they were different from *W. bancrofti* embryos, and could be assigned to a new species. Whether there is enough difference to create a new species may be argued by some and perhaps variety or subspecific rank would be sufficient, e.g., *Wuchereria bancrofti malayi*, such a trinomial name being valid for subspecies.

Another very important point, however, arises: what was the filarial embryo found by ASHBURN and CRAIG in the Philippines in 1906? I criticised (Low, 1909) the view that this was a new species, and further work supported this opinion as adults found later were found to be morphologically identical with those of *W. bancrofti*. Yet as the adults and embryos of the Pacific form have also been shown to be identical with those of *W. bancrofti*, ASHBURN and CRAIG's specimens may, and in fact very likely were, identical with the Pacific form, especially as they were definitely non-periodic. MANSON-BAHR, on the other hand, suggests that they might have been the embryos of *W. malayi*, even though they were known to be non-periodic. This raises a further difficulty, because if MANSON-BAHR's suggestion is correct, then the name *W. malayi* would have to pass into the synonymy of *W. philippinensis*. On the other hand, if ASHBURN and CRAIG's embryos and those of the Pacific form belong to one species, then, if any specific or subspecific name is to be used for the Pacific form, it would have to be *W. philippinensis* or *W. bancrofti philippinensis* respectively.

However, I do not consider there is any justification at the present time for giving the Pacific form specific or subspecific rank. It must only, I think, be considered a biological or physiological strain or race of *W. bancrofti* changed as regards its biology as P. A. BUXTON (1928) suggests.

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For further references *vide* MANSON-BAHR's paper *loc. cit.*

## CORRESPONDENCE.

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### RED PALM OIL.

*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

Two references have recently occurred in these TRANSACTIONS to the use of red palm oil in native dietaries, WILLIAMS (1940) and MOORE (1940a); a more recent paper by MOORE (1940b) refers to the vitaminization of arachis or other edible vegetable oils\*. Oil palms occur under indigenous conditions on the eastern littoral of Lake Tanganyika, and sporadically elsewhere. A small plantation exists at Tabora and a larger one with a factory producing up to 40 tons of oil a year in the Usambara Mountains near Tanga on the East Coast.

Palm oil is not a normal ingredient of native diets in Tanganyika, but for some years past the Medical Department has attempted to popularize the use of crude palm oil, ample supplies of which exist in the Congo. The quality of the palm oil purchased for use in Government institutions is now carefully controlled by specification which requires the oil to have an acid value not exceeding 15 and a colour not less than that produced by dissolving 500 micrograms of beta carotene in 1 gramme of colourless coconut oil.

Vitamin A deficiency is fairly common in Tanganyika and in the past outbreaks of night blindness in institutions such as prisons have been frequently reported. A half ounce of palm oil daily is issued to most African prisoners throughout the Territory and its introduction has been followed by beneficial effects, night blindness amongst prisoners now being almost unknown. Some sisal estates have adopted the policy of making a free daily issue to their labour of palm oil and many boarding schools and hospitals are also utilising the oil. Definite complaints from prisoners as to the use of palm oil have been traced to use of highly acid oil and the general impression is that although prisoners prefer groundnut oil they soon become accustomed to the flavour of palm oil. On estates, some labourers have declined the free issue and generally it may be said that crude palm oil is not accepted with relish, particularly at first.

\* MOORE, D. FITZGERALD. (1940a). Pellagra and red palm oil. (Correspondence.) *Trans. R. Soc. trop. Med. Hyg.*, 34, 231.

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Recently the question of a supply of vitamin A for African troops came up for consideration, and although crude palm oil was tried there was some doubt as to whether it was actually being consumed by the troops. The flavour of the palm oil was therefore removed by treatment with steam in vacuum and using the quality of crude oil specified above, deodorization was found to be almost complete after 2 to 3 hours at 150° C. Using a half-ton deodorizer, the gross loss on treatment was approximately 5 per cent., made up as follows :—

Water in crude oil .. .. .	1.5 per cent.
Carried over mechanically or volatile and recovered .. .. .	2.6 „
Net loss .. .. .	0.9 „

The cost of deodorization was less than £10 per ton of product, a figure which allows for loss. Very little carotene was oxidized during the process and spectroscopic examination of the resulting oil did not show any differences from the crude oil regarding the position of the absorption bands. Similarly no appreciable difference was found on chromatographic absorption of the treated and untreated oils but part of the free fatty acids was distilled off during the process. The treated oil was somewhat lower in price than the local prices of crude groundnut and crude white sesame oils.

The deodorized palm oil was then mixed with five volumes of good quality (edible) crude groundnut or sesame oils or with five parts of deodorized coconut oil. The mixtures appear to be well liked by African troops, who had in the past been receiving ghee (99 per cent. butter fat), much of which was of low quality. It appears to be quite stable and the mixture contains from 1 to 3 per cent. of free fatty acids calculated as oleic acid. Strict chemical control is exercised over the manufacture and carotene in the mixture is determined by a Pulfrich photometer using an S.47 filter. From 2,000 to 4,000 micrograms of carotene are present in one ounce of the mixture which has a guaranteed minimum content of 2,000 micrograms per ounce. Already several hundred tons of the mixture have been prepared.

It seems that the mixture of oils can be marketed at a price which compares favourably with other edible oils used by the native population. Crude coconut oil is, of course, much cheaper than the mixture, but this oil, the flavour of which is unpleasant, is not much used by the population except at the coast. Deodorization of red palm oil under carefully controlled conditions thus appears to be a step forward in the development of a cheap and palatable source of vitamin A for use in tropical countries.

I am, etc.,

Dar-es-Salaam.

L. D. RAYMOND,

*Government Analyst.*

## THICK FILM APPEARANCE OF MALARIA PARASITES.

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

Dr. J. W. FIELD has filled a long felt need in his series of papers on the thick film appearance of malaria parasites, and both those who have a special interest in malaria, and general practitioners in the tropics, are greatly in his debt. But, for the sake of those who may read his latest paper as they commence practice, I feel that some qualification is required of one or two of the points which he makes.

In East Africa, and I make no suggestion that the following remarks apply elsewhere, the figures given in his Table I would not be found in the course of routine diagnosis.\* In other words, parasites are likely to be just as scanty in an early subtertian infection as in any other, and diagnosis of cases in Europeans has frequently to be made on the presence of two or three parasites in a thick film.

When parasites are present in such small numbers, species diagnosis in thick films does, as Dr. FIELD himself states, become almost impossible except to the very expert, and recourse must necessarily be made to the thin film.

In the early stages of an infection with *P. falciparum*, the parasites are so minute that they may only be seen with difficulty in a thin film, and when this is the case I have on a number of occasions been unable to find them in a thick film. It is in fact just such cases which, in my opinion, provide an explanation of a number of those "negative" blood films which are found in the obvious presence of clinical malaria.

I do not, however, wish to suggest in any way that the thick film technique is not the method of choice in the diagnosis of malaria, and the mainstay of malaria survey work.

Yours faithfully,

Nairobi.

D. BAGSTER WILSON,

Major, E.A.A.M.C.

\* FIELD, J. W. (1941). The morphology of malarial parasites in thick blood films. Part IV. Identification of species and phase. *Trans. R. Soc. trop. Med. Hyg.*, 34, 405.



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COMMUNICATIONS.\*

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PLAGUE IMMUNIZATION WITH LIVE VACCINE IN SOUTH AFRICA.

BY

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In previous work in collaboration with J. H. PIRIE (PIRIE and GRASSET, 1938, 1941), we demonstrated the considerably higher degree of immunity conferred by live avirulent plague vaccine as compared with killed organisms, both in experimental immunization and in the production of a potent therapeutic serum. These experiments, which confirm the findings of GIRARD (1935) and GIRARD and ROBIC (1934, 1936) in Madagascar, and of OTTEN (1933, 1936, 1938) in Java, also illustrate the non-pathogenicity of these

\* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

†We wish to acknowledge our thanks to Dr. PETER ALLAN, Secretary for Public Health in the Union, for his authority and facilities granted for this work and for the data obtained from medical officers of his Department. We are particularly indebted to Dr. G. W. GALE, Assistant Health Officer, for the personal interest he has taken in these field investigations and for the valuable information supplied in his report; also to Dr. S. GOLDBERG, District Surgeon, for the information kindly supplied regarding the Viljoenskroon outbreak, and to Dr. J. F. MURRAY, officer in charge of plague diagnosis at the South African Institute for Medical Research, for his much appreciated co-operation in these investigations.

spontaneously attenuated, though highly antigenic, plague strains for experimental animals. Massive doses, such as a whole agar slope of the avirulent selected strains ("E.V.," Madagascar; "Tjiwidej," Java; and "No. 2," South Africa) can be injected into guineapigs, rabbits, and white and wild rats without producing any untoward morbid symptoms.

Convinced of the harmlessness and efficacy of the method, and encouraged by the most gratifying results obtained by large scale immunization with live vaccine in Madagascar and Java, in over four million persons over a period of 6 years, we felt justified in proceeding with the application of the method to human immunization in South Africa.

In Madagascar plague immunization with the live avirulent E.V. strain was introduced by G. GIRARD, Director of the Antananarive Pasteur Institute, in 1933, and has been applied to over 800,000 persons during the period 1933-36 without any accidents. This extensive experiment resulted in an annual reduction of the plague incidence in endemic areas by 80 per cent., as compared with previous years during which various kinds of killed vaccines had been used. (GIRARD, 1935, 1939; GIRARD and ROBIC, 1938; LEAGUE OF NATIONS, 1937). The death rate was reduced by one-third of the rate in the uninoculated. Finally, not a single case of pneumonic plague, primary or secondary, was observed among the population vaccinated with the live vaccine.

Similarly, in Java, large scale immunization with live plague vaccine, using the local Tjiwidej strain which had also spontaneously become avirulent, although retaining high immunizing properties, was introduced by L. OTTEN, Director of the Bandoeng Pasteur Institute. Over two million persons were inoculated during the period 1935-36 with this live vaccine. (OTTEN, 1936, 1938; LEAGUE OF NATIONS, 1937). The plague incidence rate was reduced by 82 per cent. within the 6 months following the introduction of mass inoculation. In the districts where alternative immunization was organised in 1934 the mortality rate amongst the inoculated population proved to be five times lower than among the controls.

#### IMMUNIZATION WITH LIVE PLAGUE VACCINE IN SOUTH AFRICA.

A preliminary series of immunization with live plague vaccine was carried out in 1940, on volunteers among members of the staff of this Institute, using E.V. (Madagascar) or Tjiwidej (Java) strains, and starting with bacterial concentrations varying from 100 to 500 million organisms.

The vaccinal emulsion consisted of 24 hour agar growth at 37° C., so adjusted as to contain 1,000 million organisms per c.c. Bacteriological controls for purity were carried out systematically from the vaccinal emulsion and from the secondary growth obtained from the latter. Animal controls were also done for each batch of vaccine; guineapigs and white rats were injected subcutaneously with doses varying from 4,000 to 20,000 million organisms,

and were kept under close observation. The vaccine was not used before the 5th day, thus assuring the necessary period for bacteriological and avirulence controls.

The vaccinal emulsion was kept in the ice chest at  $+2$  to  $4^{\circ}$  C., and used from the 5th to the 15th day. Secondary medium planting with decreasing dilutions of the bacterial suspensions (from 1 in 10 to 1 in 100,000) have shown that no appreciable deterioration of the vitality of the vaccine takes place during this period, under such storing conditions.

### *Technique of Immunization.*

In all cases the inoculation was made subcutaneously in the postero-inferior area of the arm, above the elbow. Full clinical data were taken for each individual, including temperature and pulse rate, before inoculation and 24 and 48 hours, up to 4 days after the inoculation. Altogether fifty persons were submitted to these preliminary tests, including four Europeans (two males and two females) and forty-six adult male natives.

### *Vaccinal reactions.*

No apparent symptoms, malaise or rise in temperature were observed after injections varying from 100 to 500 million live organisms. The immunization dose was therefore gradually increased to 1,000 million in a volume of 1 c.c.

*Local reactions*, following the inoculation of 1,000 million of either E.V. or Tjiwidej vaccinal emulsion were limited to slight transitory inflammation with some tenderness at the site of the injection. In a few persons a larger oedematous patch was observed, which faded within 48 hours.

*General reactions.* These were exceptional and moderate. Three of the persons injected with 1,000 million Tjiwidej reported some lassitude with headache during the 36 hours following the inoculation. Temperature and pulse remained normal in all immunized persons, with one exception—a slight rise (99.6) the day after the vaccination.

On the whole no appreciable differences in the nature of the reactions could be observed after E.V. or Tjiwidej inoculation.

Similar clinical data confirming the harmlessness of the method were collected in a third series of immunizations, using as antigen a South African avirulent plague strain—No. 2. As reported in a previous publication with J. H. PIRIE, this strain, isolated in Pietermaritzburg in 1904, has been subcultured in this Institute ever since, and has been found by trial to be avirulent. In comparative protection tests on rats with E.V. and Tjiwidej strains, No. 2 has also been shown to possess high immunizing properties.

Cultures from blood of immunized subjects at different intervals during

the 48 hours following vaccination with these live strains, and incubated at 27° and 37° C., always remained negative.

As the result of the preliminary series, it was decided to adopt, as the standard immunizing dose, a concentration of 1,000 million live organisms consisting of 500 million E.V. and 500 million Tjiwideoj organisms. Immunological experiments have shown that the E.V. strain gives a higher degree of protection in the guineapig than the Tjiwideoj strain; on the other hand, in the rat the Tjiwideoj strain confers a higher immunity than the E.V. strain. OTTEN, after studying several avirulent plague cultures, is of the opinion that these results indicate the presence of at least two types of antigens present to different extents in the various strains. With regard to human immunization, as we are not in a position to compare immunity in man with that in guineapigs and rats, the most beneficial procedure would appear to be the use of a mixed vaccine prepared from the main types of strains, such as E.V. and Tjiwideoj, as suggested by OTTEN.

#### SEROLOGICAL TESTS ON BLOOD OF IMMUNIZED SUBJECTS.

1. *Agglutination.* Samples of blood of persons vaccinated with a single injection of Tjiwideoj or E.V. strain, or a mixture of the two, were taken from 32 to 42 days after inoculation. Comparative agglutination tests were carried out against live and heated emulsions of E.V., Tjiwideoj and heated emulsions of virulent plague strains. Readings were done after 2 hours of contact at 37° for the live emulsion and 52° for the heated ones, and also after a further period of 18 hours at room temperature for both series.

When present, agglutination was quite definite, but it remained, on the whole, at a very low titre: 1 in 20 for most of the cases to 1 in 80 for one case only. Guineapigs and rats immunized with the same dose of live vaccine also gave low agglutination rates, 1 in 20 to 1 in 40. In comparison sera from horses hyperimmunized with live E.V. and Tjiwideoj strains agglutinate the above-mentioned strains to titres of from 1 in 400 to 1 in 800.

2. *Precipitin tests* were carried out with the same sera. The antigen consisted of an autolysate obtained from concentrated plague emulsion, submitted to repeated low freezing and thawing, according to the technique described by the author for the preparation of *B. typhosus* endotoxin. Tests carried out with various concentrations of antigen and dilutions of sera all led to negative results. In comparison a rapid precipitation is observed with the undiluted antigen in contact with sera of immunized plague horses to titres such as 1 in 800.

#### IMMUNIZATION IN THE MOROKWEN RESERVE PLAGUE OUTBREAK.

At the beginning of January, 1941, seven successive deaths, attributed to plague, were reported among the native population of a village in the Morokwen Reserve (in the Kalahari, near the Bechuanaland border of the Cape Province).

Postmortem examination by the District Surgeon of Vryburg, after exhumation of two of the bodies, led to the provisional diagnosis of pneumonic plague, which was confirmed bacteriologically by the culture of virulent *Bacillus pestis*, isolated by Dr. J. F. MURRAY from postmortem material sent to the South African Institute for Medical Research.

Dr. G. W. GALE, Health Officer charged by the Public Health Department to deal with the outbreak, found on his arrival several new cases, all of whom died of pneumonic plague. It was consequently decided to proceed with anti-plague immunization among the contacts and the native population of the affected area. Although the pneumonic type of the outbreak was not the most suitable for assessing the protective value of a plague vaccine, it was agreed to use the new live vaccine instead of the heated phenolized vaccine hitherto employed, and of which a first dose had been given to a small number of persons at the beginning of the outbreak.

From the data contained in the report kindly supplied by Dr. GALE, over a thousand persons were immunized with the live plague vaccine from the 31st January to the 24th February; the majority were natives of both sexes of whom about 25 per cent. were children. Approximately fifty Europeans were also vaccinated. As the outbreak spread to several villages, the more distant being 25 miles away from the original focus, the vaccination was extended to the population of the affected districts. The vaccine was injected subcutaneously in the postero-superior zone of the arm, at the insertion of the deltoid muscle. The standard dose of 1 c.c. (1,000 million organisms) was given to all adults. Children under the age of 12 received proportionately smaller doses.

*Reactions.* No spontaneous complaints were received from the inoculated subjects. When questioned, the majority of persons had no complaints of pain or generalised reaction to report. Some stated that the site of injection had been painful and somewhat tumid during the first or second day after inoculation.

The great majority of persons immunized were certainly not direct contacts. Dr. GALE estimates that over 100 persons were certainly in close contact with certified pneumonic cases and did not themselves contract plague. Of these, however, at least twenty had been contacts a week or more before inoculation was started. One cannot therefore logically claim that the remainder necessarily owed their escape to immunization only. From 10th January to 10th February thirty-six persons died of plague, of whom five had been vaccinated.

With regard particularly to the *immunized intimate contacts*, of a total of 110, 104 did not contract plague; six contracted the infection, of whom five died of pneumonic plague and one recovered. Of this group of 104 immunized contacts who escaped, twenty were not vaccinated until 7 days or more after being in contact with infected persons, thirty-seven were vaccinated within 7 days of contact and forty-seven *before* contact. Although in



reality these figures may be higher they only include those who certainly entered patients' huts, attended known cases and in many cases slept with them, or handled the bodies or intimate possessions of those who had recently died of plague.

Details of the data referring to the six immunized persons who contracted plague (all pneumonic) are shown in the following table :—

Sex	Age	Contact with a case of pneumonic plague	Onset of symptoms of plague	Day of illness on which death occurred
Male	65	5-6 days <i>before</i> vaccination	Same day as vaccination	1st
Male	55	5-6 " " "	2 days after vaccination	1st
Female	30	3-4 " " "	3 " " "	3rd
Female	70	0-1 " " "	3 " " "	3rd
Female	70	0-1 " " "	3 " " "	4th
Female	17	5-6 " <i>after</i> "	9 " " "	Recovered.

It can be seen that for all these cases except the one who recovered the time which elapsed between the contact and the date of immunization varied from the very same day to a maximum of 5 to 6 days. Onset of symptoms and death occurred within the days following inoculation.

From the immunological point of view the immunization, which was carried out during the period of incubation or actually at the beginning of the invasion phase in some cases, could not be expected to have much chance of controlling the rapid evolution of the infection, particularly the pneumonic type.

The above figures, nevertheless, suggest that in some cases the vaccine may, to a certain extent, have increased the resistance to infection without full protection having time to develop; *e.g.*, the two old women of 70 who were vaccinated within 24 hours of contact and who showed the first symptoms of pneumonic plague 3 days later, and only died after 3 and 4 days of illness. Of the thirty fatal cases among the *unvaccinated* several did not succumb until the 4th day, but they were mainly young adults. No case over 50 survived until the 4th day with the exception of the second old woman, who had been immunized, referred to above. An unvaccinated woman of about 70 died on the first day of illness.

As regards the last case in the table, the girl of 17—the only patient who recovered—she became a contact 5 to 6 days *after* vaccination, and although of a weak constitution she finally recovered. She was very ill; in fact, on the 2nd and 3rd day of illness her death was anticipated almost hourly. Against all expectations, she was still alive on the morning of the 4th day and was given 200 c.c. of antiplague serum intravenously. The following day she was much

better and recovered completely. [The serum used was the concentrated antiplague serum prepared at the South African Institute for Medical Research, from a number of avirulent strains of *B. pestis*, including South African strains. (PIRIE and GRASSET, 1938).] As pointed out by Dr. GALE, who attended the case personally, this history suggests that recovery was due to partial active immunity established by the vaccine, which apparently carried the patient through the crisis, for she had definitely, although only very slightly, improved before she received the serum.

The number of persons involved in this interesting immunological field experiment is too small to allow of any conclusive statistical interpretations.

#### VILJOENSKROON PLAGUE OUTBREAK.

Immunization with live plague vaccine was applied in a second small outbreak in the Viljoenskroon district (Orange Free State) during March and April, 1941.

From information kindly supplied by Dr. S. GOLDBERG, local District Surgeon, five cases of plague were observed on three farms of the district, including three cases of bubonic and two pneumonic plague, the last two being fatal.

Altogether ninety-six persons, mostly natives, were immunized with the live plague vaccine. The immunizing dose was, as previously, 1,000 million organisms in 1 c.c. Similar observations to those reported in the previous series regarding the mild nature of the post vaccinal reactions were generally noted.

No marked systemic reaction following the inoculation was recorded, except in a single complex case, that of Dr. H. L., who assisted Dr. GOLDBERG in this outbreak; he was in direct contact, and 4 days before he was vaccinated performed a postmortem examination on the body of a native who had died of bacteriologically confirmed pneumonic plague. On the 3rd day after vaccination he developed a temperature of 103°F., pulse rate 120, accompanied by rigors and pain at the site of the injection, but no glandular symptoms. These symptoms subsided within 24 hours. Dr. H. L. is known as an allergic reactor. On the other hand, this happened on the 4th day after contact during postmortem examination, and the possibility of a mild abortive form of plague, as the result of the active immunization, is not to be excluded.

A brief review of this small outbreak shows that two fatal cases of pneumonic plague, confirmed bacteriologically, were reported on the farm "Oorskief." Fifty-six persons were consequently immunized, of whom ten were direct or close contacts. In the hut in which one native died after contact with the first fatal pneumonic case, four native females—direct contacts living in the same hut—were vaccinated with 1 c.c. of the live vaccine. In spite of their intimate contact with the fatal case these four natives remained free from

plague and no further cases beyond the two original unvaccinated victims were observed in this immunized community.

A month later three bubonic cases were reported on two farms in the same area (14th and 15th April). These cases were treated with plague serum and recovered. Forty persons living on these two farms were immunized with the live vaccine. No further cases of plague occurred after immunization.

Incidentally, the therapeutic results obtained in the treatment of the bubonic cases of this series with concentrated plague serum were reported to be very satisfactory. Serum was injected intravenously in doses of 50 c.c. for 3 or 4 days consecutively and was accompanied by rapid control of the infection, followed by uncomplicated convalescence.

Apart from these two outbreaks live plague vaccine has been supplied periodically, at the institution of the Public Health Department, for the immunization of small communities in several infected areas in the Union.

Routine preparation of the live vaccine has been carried out since the beginning of 1941, at the rate of a batch every 2 to 3 weeks. Vaccination of the newcomers to the native staff of the Institute has been performed with samples of most of the batches prepared, thus providing us with a useful periodical human record. The reactions which we have thus been able to keep under observation in these cases have always been of a similar benign type to those reported above.

### CONCLUSIONS.

The plague problem in South Africa has obviously not the same importance as in plague endemic countries such as Madagascar, Java or India. The periodical outbreaks observed in this country have mostly remained limited to small epidemic foci, generally in the rural native population. The question of wholesale antiplague immunization of the native population is therefore, under present conditions, out of consideration.

The immunological data obtained from the application of live plague vaccine to those small outbreaks, although too limited to be conclusive in themselves, are nevertheless quite useful in many ways. They help us to judge at first hand the harmlessness of the method, the benign nature of the vaccinal reactions observed, and the relative degree of protection conferred by this type of vaccine against the bubonic and pneumonic forms of the infection, and provide us with practical experience regarding the preparation, application and keeping properties of the vaccine, should the necessity arise to use it on a large scale.

From the practical and administrative point of view, immunization with live plague vaccine has the great advantage of requiring only a single injection, instead of two with the killed vaccine, thus allowing mass inoculation to be carried out in a much shorter time, should an epidemic occur. Its employment

is simple and without danger, being accompanied by little pain and reaction, and it is well accepted by uneducated native populations.

From the economical point of view the method requires considerably less immunizing material and a simpler medical and health organization, and is therefore more economical.

Following inquiries received from a number of African Government Institutions responsible for the production of plague vaccine, and requests for strains of *B. pestis* used in this Institute for the production of live vaccine, we thought that the information contained in this paper might, under the present circumstances, eventually be of some use to those interested in this new form of prophylaxis.

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PNEUMONITIS IN MICE.  
INFECTED INTRANASALLY WITH Q FEVER.

BY

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Within the past few years a number of observers have studied the effects of instilling rickettsiae intranasally in rodents. SPARROW and LUMBROSO (1929) inoculated guineapigs and rats intranasally with exanthematic and murine typhus rickettsiae, no anaesthetic being used. Attenuated generalized infections were set up but no attempt was made to obtain localized pulmonary lesions by massive intranasal inoculation. Later OKAMATO (1937), after giving intraperitoneal injections of murine rickettsiae to mice, observed rickettsiae in the endothelial cells lining the pleural cavities and in the alveolar epithelium of the lungs. These cells appeared to be more heavily infected than the cells of either the spleen or liver. WOHLRAB (1937), also using the murine type of rickettsia, inoculated mice by a variety of routes, including the intranasal, when the animals were under light ether anaesthesia. Rickettsiae were found within the endothelial cells of the pleura but no reference is made to any pneumonic process within the lung. HIRTZ (1938) is said to have grown *Rickettsia prowazeki*, *in vitro*, in minced guineapig lung tissue suspended in ascitic fluid. CASTANEDA (1939), however, was the first to study the pneumonic process set up by intranasal instillation of rickettsiae. Rickettsiae of an orchitic strain L were instilled into the nares of mice, rats and rabbits. Mice were found to die, in the majority of cases, within 96 hours, while rats were more resistant when the intranasal inoculum was derived from the tunica vaginalis

of guineapigs. Rats, mice and rabbits showed the most extensive lesions when infected mouse lung was used as an inoculum, death taking place in from 72 to 96 hours.

More extensive experiments were carried out by DURAND and SPARROW (1940), the rickettsiae both of endemic and exanthematic typhus as well as of fièvre boutonneuse being inoculated intranasally into mice under ether anaesthesia. Later DURAND, GIROUD and SPARROW (1940) obtained pulmonary lesions by instillation of the rickettsiae of Rocky Mountain spotted fever.

In the present communication the results of inoculating the rickettsia of Q fever into mice intranasally are described. While these experiments were in progress evidence was brought forward that the American form of Q fever is capable of setting up a form of pneumonitis in man. An outbreak of fever associated with pneumonitis or central pneumonia occurred in the National Institute of Health at Washington, D.C. Altogether, according to HORNIBROOK and NELSON (1940), there were fifteen proven cases of infection among 153 persons employed in one building, although curiously enough there were no cases of infection in the wing of the building where work had been in progress on Q fever since the spring of 1938. From the blood of certain of these cases DYER, TOPPING and BENGSTON (1940) were able to isolate a rickettsia which was identical with that of Q fever, while during convalescence there developed in the blood of the patients immune bodies capable of neutralizing the rickettsiae of Q fever. There was no evidence that any arthropod vector was in any way responsible for the transmission of the disease or that the disease was spread by personal contact though there is a possibility that the rickettsiae may have been inhaled in the form of dust. Only one case was fatal, the postmortem results being described by LILLIE, PERRIN and ARMSTRONG (1941) who at the same time set up a pneumonitis in rhesus monkeys by intrapulmonary injection of the rickettsia of Q fever. In addition to the human cases associated with pneumonitis occurring in Washington, HESDORFFER and DUFFALO (1941) have described two patients with Q fever from Montana. The patients had been out in the woods but as one case occurred in the late fall in October, the other at Christmas, it is most unlikely that any ticks were active at that time. Both patients denied having been bitten by ticks. A number of other cases of pneumonitis have been described in the United States and in Hawaii; certain of these may have been due to Q fever.

#### EXPERIMENTAL TECHNIQUE.

Two strains of the rickettsia of Q fever were employed in these experiments, the original Q strain isolated by BURNET and FREEMAN in Australia in 1937 and the X strain obtained from the tick *Dermacentor andersoni* collected near Nile Mile Creek in Montana in 1938 by DAVIS and COX and subsequently studied by COX (1938). I have to thank Dr. F. M. BURNET for his kindness in supplying me with the two strains of rickettsia. Both Australian and American workers are now agreed that Q fever as it appears in the

two continents is identical and that the rickettsiae responsible are strains of one and the same organism. The rickettsia was named by DERRICK *Rickettsia burneti* on 7th January, 1939, while subsequently on 6th October, 1939, COX proposed the name *Rickettsia diaporica* for the rickettsia of Q fever in America. If it is agreed that the two rickettsiae are strains of the same organism *R. burneti* obviously has priority.

Material for the primary intranasal instillation was derived from mouse spleen. As BURNET and FREEMAN (1937) have pointed out, intraperitoneal injection of mice with *Rickettsia burneti* produces an inapparent infection, but the spleen, more especially from the 7th to the 10th day after inoculation, is very rich in rickettsiae. The infected spleens were ground up in physiological saline to make a 10 per cent. suspension: this was lightly centrifuged for 10 minutes at 3,000 r.p.m. and four drops of the supernatant were instilled into the nostrils of white mice under ether anaesthesia: for subsequent passages mouse lung suspensions were similarly prepared: four mice were inoculated at a time. Swiss mice of not more than 20 grammes body weight were employed.

#### EXPERIMENTAL RESULTS WITH *RICKETTSIA BURNETI*.

Similar results were obtained with both the Australian and American strains. Four passages were made with the former, eight with the latter. Except in two instances the infection was not fatal to mice. Mice were killed in from 4 to 10 days after instillation. Macroscopic examination of the lungs showed small areas of consolidation distributed somewhat irregularly throughout the lobes. Sections of lung were stained with iron alum and haematoxylin, with Maximow's stain and by Giemsa's method. Smears from the lungs were treated with Macchiavello's stain, Castaneda's and Giemsa's method and by the Feulgen technique. The histological changes within the lung were as follows. The areas involved show a somewhat nodular arrangement in association with terminal bronchi or bronchioles. In the bronchi there is found a varying amount of fibrinous deposit together with a cellular exudate composed very largely of round cells, some with small deeply staining nuclei, other larger mononuclear cells with kidney shaped nuclei and a few with the plasma cell type of nucleus. In addition a good deal of nuclear debris is also present. The cells lining the bronchi are prominent but in places have undergone considerable desquamation (Fig. 1). Many of the alveoli are filled with sero-fibrinous exudate staining red with eosin (Fig. 2) but containing few, if any, cells: in these areas there is only a very slight increase in the number of cells in the interstitial tissues. In some alveoli, however, the sero-fibrinous deposit contains a few mononuclear cells while in other areas there is almost complete obliteration of the alveolar spaces (Fig. 3). The obliteration appears to be due almost entirely to the increased interstitial reaction. The cells lining the pulmonary alveoli are swollen while the interstitial tissues are crowded with the same types of cells as are present in the bronchial exudate, small lymphocytes, large mononuclears and a few plasma cells. Some increase in fibroblasts appear in certain areas in the alveolar walls. In addition a few polymorphonuclear leucocytes are also present in the interstitial tissues and here and there are small nodules composed of dense masses of lymphocytes and mononuclear cells with some polymorphonuclear leucocytes (Fig. 4). Much nuclear debris



is also present in these nodules. The blood vessels in the alveolar walls are congested and contain many mononuclear cells: the epithelium lining the capillaries is swollen but haemorrhages are not seen. The cells lining the pleural surface of the lung are prominent and at some points appear to have undergone proliferation (Fig. 5). In many of these endothelial cells the nuclei stain poorly. An occasional basophil and in one mouse a few eosinophils were seen among the proliferated cells of the pleura while the alveoli subjacent to such areas are filled with fibrin. In smears from the lungs stained by Macchiavello's method rickettsiae can be seen in large numbers (Fig. 6) either free or in close association with mononuclear cells. In sections stained by Giemsa's method rickettsiae were present in large mononuclear cells on the pleural surface of the lungs, in alveoli and in the lumen of the bronchi.

These changes are very similar in character to those recorded by LILLIE and his colleagues (1941) in the fatal case that occurred at the National Institute of Health. The patient was a male aged 59. Macroscopically, the right lung showed congestion in the lower lobe while the upper lobe exhibited firm grey granular consolidation. Congestion and oedema were noted in the left lung, more especially in the lower lobe. Microscopically the changes in the consolidated area resembled those in mice except for the occasional presence of numerous red cells in scattered alveoli.

In monkeys injected by LILLIE and his colleagues directly into the right lung the amount and extent of consolidation were variable. Consolidation is nodular or confluent nodular in type and generally peribronchial or peribronchiolar in location. The pleura over consolidated areas, focally elsewhere and about the hilus, is described as showing patches of stratifying mesothelial proliferation with infiltration by polymorphonuclear leucocytes and occasionally eosinophil leucocytes.

The lesions in the human case and in the monkeys were very similar to those recorded by KNEELAND and SMETANA (1940) and by LONGCOPE (1940) in patients dying with bronchopneumonia of unknown aetiology.

#### PNEUMONITIS IN MICE FOLLOWING INTRANASAL INSTILLATION OF EXANTHEMATIC AND MURINE TYPHUS RICKETTSIAE.

In view of the changes produced in the lungs of mice by the rickettsiae of Q fever a brief description of the lesions set up in the same species by intranasal instillation of exanthematic and murine rickettsiae may not be without interest. The strains of rickettsiae employed were Nicolle's strain of exanthematic typhus and the murine rickettsia No. 1 of Tunis isolated by Madame HÉLENE SPARROW. My thanks for these strains are due to Dr. PAUL DURAND and Madame HÉLENE SPARROW, of the Pasteur Institute of Tunis, and Dr. PAUL GIROUD, of the Pasteur Institute, Paris.

Brief reference to the histological changes found in the lungs of mice infected intranasally with an orchitic strain from Mexico has been made by

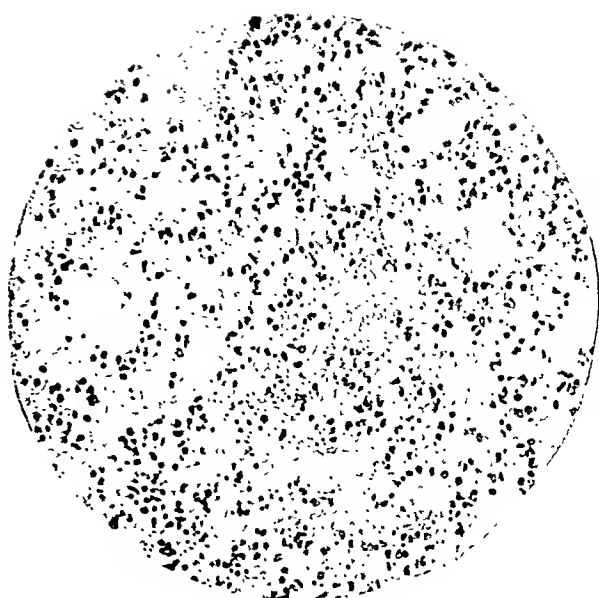
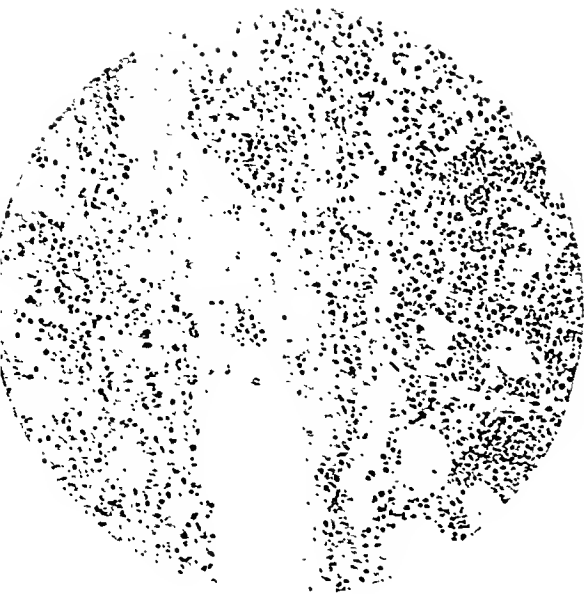


FIG. 1.—Lung of Mouse with Q fever (American strain). Bronchus filled with exudate : desquamation of epithelial lining. *Stained iron alum and haematoxylin.* ( $\times 175$ ).

FIG. 2.—Lung of Mouse with Q fever (American strain). Pulmonary alveoli filled with sero-fibrinous exudate. *Stained iron alum and haematoxylin.* ( $\times 200$ ).

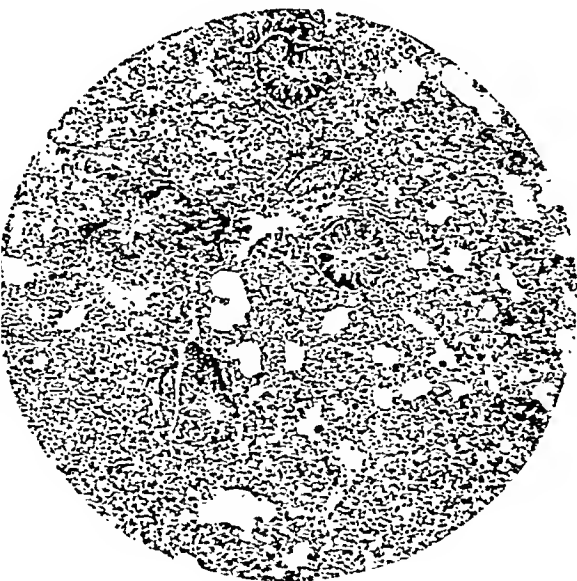


FIG. 3.—Lung of Mouse with Q fever (Australian strain). Interstitial infiltration and proliferation with occlusion of many alveoli. *Stained iron alum and haematoxylin.* ( $\times 75$ ).

FIG. 4.—Lung of Mouse with Q fever (Australian strain). Interstitial changes, with a nodule of mononuclear cells and polymorphonuclear leucocytes. *Stained iron alum and haematoxylin.* ( $\times 175$ ).



FIG. 5.—Lung of Mouse with Q fever (American strain). Proliferated endothelial cells on the pleural surface of the lung : the alveoli immediately beneath are filled with fibrinous exudate. *Stained iron alum and haematoxylin.* ( $\times 175$ ).

FIG. 6.—Smear from lung of Mouse with Q fever showing rickettsiae. *Stained Machiavello's method.* ( $\times 1500$ ).

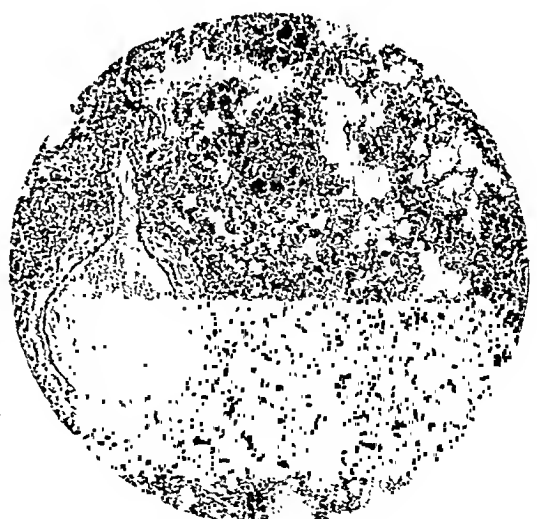
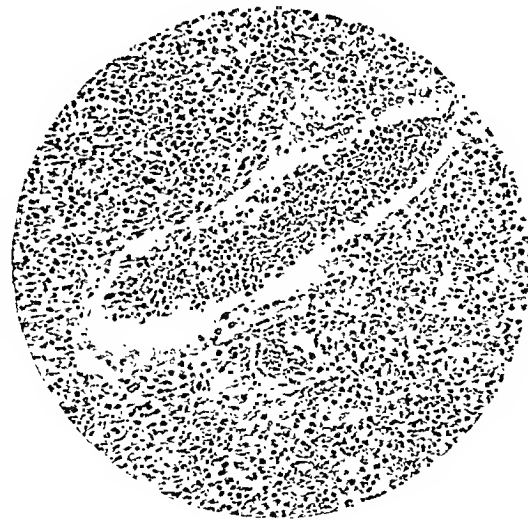


FIG. 7.—Lung of Mouse with exanthematic typhus : plugging of bronchus with cellular exudate ; infiltration and proliferation into the interstitial tissue. *Stained Maximow's method.* ( $\times 175$ ).

FIG. 8.—Lung of Mouse with murine typhus : interstitial infiltration with obliteration of some alveoli ; serous exudate in other alveoli : congestion of blood vessels. *Stained iron haematoxylin and eosin.* ( $\times 65$ ).

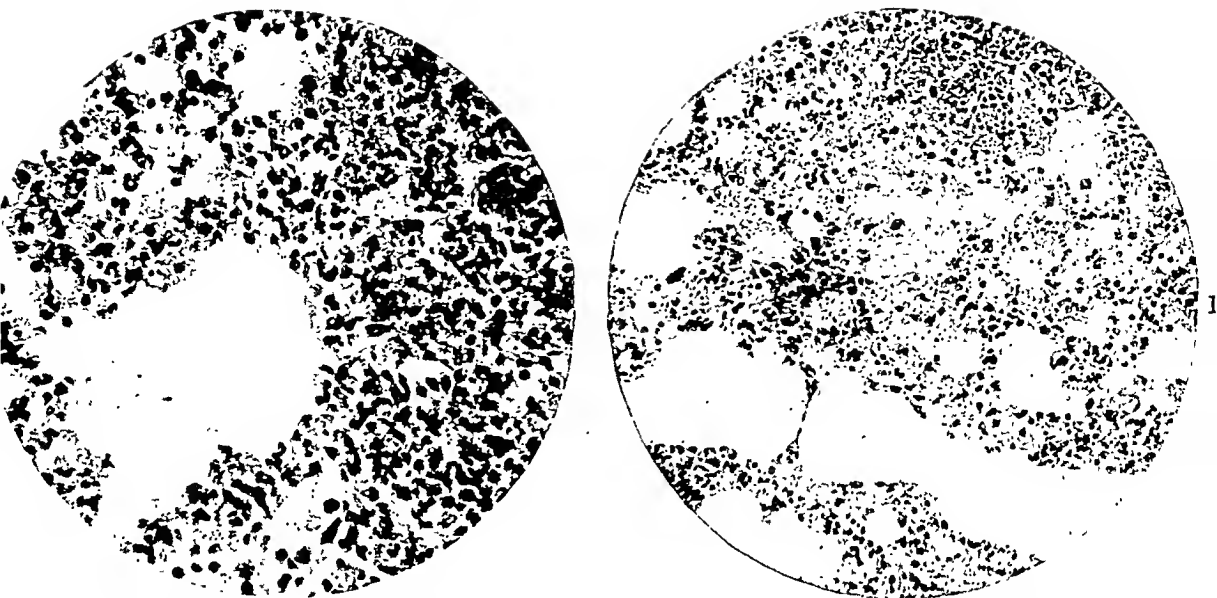


FIG. 9.—Lung of Mouse with murine typhus : interstitial infiltration of interstitial tissues with mononuclear cells and polymorphonuclear leucocytes : small extravasations of red blood corpuscles. *Stained iron haematoxylin and eosin.* ( $\times 175$ ).

FIG. 10.—Lung of Mouse with exanthematic typhus : interstitial infiltration, with nodules of cells ; serous exudate in some alveoli. *Stained iron alum and haematoxylin.* ( $\times 175$ ).

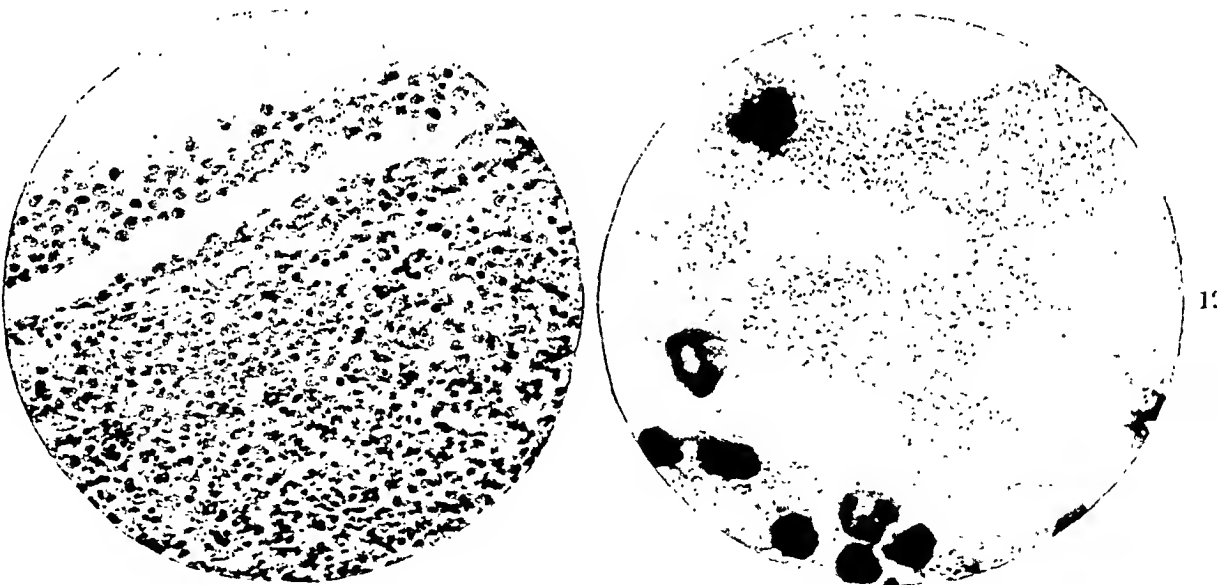


FIG. 11.—Mouse lung exanthematic typhus : proliferation of cells on the pleural surface. *Stained iron alum and haematoxylin.* ( $\times 250$ ).

FIG. 12.—Smear from lung of Mouse with exanthematic typhus showing rickettsiae. *Stained Machiavello's method.* ( $\times 1000$ ).

## CONCLUSIONS.

Intranasal instillation of the rickettsia of Q fever, both Australian and American strain, in mice causes an interstitial pneumonia. The lesions are similar in character to those induced in mice by the intranasal instillation of the rickettsiae of exanthematic and murine typhus.

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## A NOTE ON BACTERIA-FREE CULTURES OF *TRICHOMONAS* *HOMINIS*

BY

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For obvious reasons the number of *Trichomonas* species cultured without bacteria is small. Up to the present pure cultures of *T. foetus* (Riedmuller) were obtained by WITTE (1933) and *T. columbae* (Rivolta) by BOS (1933) from sterile pus containing the flagellates and *T. eberthi* by TENNENBAUM (1939) from an explantate of a spontaneous leukaemia tumour in a fowl. GLASER and CORIA (1935) separated *T. foetus* from contaminating bacteria in the vaginal washings of infected cows by mechanical means; the infected material was carefully placed on the surface of a semi-solid medium contained in a V tube and the flagellates penetrated through the medium to the other side of the V tube more rapidly than the bacteria and were then sown free from bacteria on Kofoed and Wagner's medium.

CAILLEAU (1939) obtained pure cultures of *T. gallinarum* from turkeys by treating cultures with various antiseptics.

A culture of *T. hominis* was made directly from human faeces on semi-solid medium previously inoculated with *B. prodigiosus*. The composition of the medium was as follows:—

Agar (3 per cent.)	...	...	...	...	1 part
Locke (containing 0.1 per cent. glucose)	...	...	...	...	8 parts
Inactivated goat serum	...	...	...	...	1 part

Rice starch is added to the medium.

The flagellates were maintained on this medium for a year, subculturing every 10 days. Attempts to obtain bacteria-free cultures by adding various antiseptics or by isolating individual flagellates all failed, but the following simple combination of mechanical and biological methods quickly gave successful results.

A V tube about 6 mm. diameter containing fine sand to a level of about 1 to 2 cm. above each bend is sterilised. A solution of 1:20,000 or even 1:10,000 gentian violet in Locke solution is added on one side of the V tube; the whole sand is quickly moistened by the solution and the bulk of the gentian violet is absorbed by the upper layer of the sand on the side to which the solution was added. A rich culture of *T. hominis* swarming with bacteria is added to the other side of the V tube; the upper level of the culture should be at least 1 cm. higher than the solution on the other side. The V tube is then placed overnight in the incubator at 37° C. On the following morning the side to which the solution of gentian violet was added is examined and some flagellates with none or very few bacteria are found in all layers of the fluid. The flagellates pass through the sand, including the layer impregnated with gentian violet, much more rapidly than the bacteria.

The fluid from the arm of the V tube to which the gentian violet was added is then sown on an egg slant covered with semi-solid medium of the formula given above and rice starch added.

In cases where the fluid sown obviously contained a few bacteria rich contaminated cultures of *T. hominis* were obtained. In cases where the fluid appeared to contain only flagellates either no cultures or contaminated cultures were obtained. We therefore suspected that the cultures had become contaminated by a few bacteria which the flagellates had ingested and transported through the sand.

We then adopted the following technique: either the fluid containing flagellates free or almost free from bacteria was sown on the above medium to which was added a 1 c.c. of a suspension of prontosil rubrum (1 gramme to 10 c.c. sterile water; the suspension is boiled and cooled before use); or the culture was allowed to grow for a day and the prontosil then added. Very rich cultures of *T. hominis*, together with a few bacteria, are obtained. The prontosil limits the number of bacteria, and though readily ingested by the flagellates does not prevent their rapid multiplication. A subculture on the above medium (together with prontosil and rice starch) is made, and when this is teeming with flagellates 1 c.c. is inoculated intraperitoneally into a mouse. On the following day the mouse was sacrificed and the peritoneal fluid was found to contain leucocytes and numerous active flagellates, many of them containing granules of starch or prontosil. The inoculated starch promotes a leucocytic response which destroys the few bacteria inoculated.

The peritoneal fluid of the mouse is sown on egg slants covered with the above described semi-solid medium with the addition of a piece of sterile mouse

liver or a little ascorbic acid or a drop of lemon juice sterilised by filtration. On this medium *T. hominis* produces rich cultures which live up to 7 days. Up to the present we have obtained twenty bacteria-free subcultures. The flagellates multiply rapidly between pH 6.6 and 7.2 and the medium becomes progressively acidified with the growth of the flagellates. We observed no multiplication at pH 7.5 and in order to obtain constantly good results the initial pH of the medium inoculated should not be above 7.3.

#### SUMMARY.

A simple method for obtaining bacteria-free cultures of *Trichomonas hominis* is described.

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## HEIGHTS AND WEIGHTS OF CANTONESE ADULT MALES.

BY

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Records dealing with the heights and weights of the Chinese of South China are few; the author has not met with any extensive one for Cantonese. The material presented in this paper is based on an analysis of the measurements taken during the routine examination of over 11,800 adult males admitted to the Hongkong prison during the years 1936 to 1937. A considerable amount of selection had to take place in order to be sure that statistics dealing only with healthy Chinese were used.

Prisoners on admission to the prison are examined by the medical officer and, according to his findings, are grouped as "hard labour," "half labour" or "unfit" according as they are found to be fit, fair or unhealthy. In so far as men under 50 years of age are concerned, only able bodied adults graded as "fit" were accepted in this series. In the prison routine no prisoner aged 50 years or over was grouped as fit for hard labour, his history sheet would be marked as "half labour" or "unfit." All such case sheets, therefore, had to be carefully scrutinised to determine whether or not the man could be accepted as healthy enough for my purpose. In many cases it was found to be very difficult to know if the case was in all respects comparable with those in younger age groups marked as being fit, and it is therefore necessary to accept with a certain amount of reserve the data concerned with those of 50 years and over in the following tables. With such a proviso, however, the author believes that the tables represent information collected from a very

\* My thanks are due to Dr. A. H. BARWELL, Medical Officer of the Hongkong prison, for his permission to use the data on prisoners' records, and to Dr. P. S. SELWYN-CLARKE, Director of Medical Services, Hongkong, for his permission to publish this paper.

average sample of the healthy lower class Cantonese male population of the Colony.

The prisoners are mainly from the urban areas, but perhaps 5 per cent. or less come from the rural districts or from the junk population of the waters around Hongkong.

According to the census returns (UTTLEY, 1938) only 30 per cent. of the population claimed to have been born in the colony; this figure, which includes children, would be lower in the case of adult males. For information as to the degree to which Hongkong population is a migrant one, and for other factors affecting it, the reader is referred to the paper already mentioned. Chinese, not natives of Kwangtung *i.e.*, not of Cantonese race, were not accepted in this series.

All weights were read to the nearest lb. and all heights to the nearest  $\frac{1}{4}$  inch. Chinese reckon their ages in a manner different from Europeans; in

TABLE I.  
CANTONESE MALES. MEAN HEIGHTS AT DIFFERENT AGES.

Age group in Years.	Number in Group.	Mean height in Inches.	Standard deviation of the Height.
16	373	62.25	1.34
17	397	62.89	2.01
18	294	63.55	2.14
19	481	63.87	2.14
20	276	64.54	2.15
21	472	64.27	2.20
22	388	64.48	2.10
23	422	64.56	2.16
24	530	64.45	2.31
25	580	64.38	2.26
26	395	64.54	2.16
27	539	64.51	2.31]
25-29	2549	64.48	2.22
30-34	1817	64.48	2.21
35-39	1322	64.50	2.18
40-44	677	64.29	2.24
45-49	542	64.36	2.32
50-54	511	64.95	2.18
55-59	424	63.74	2.06
60 +	372	63.60	2.05
All ages ...	11,847	64.228	2.24

TABLE II.  
CANTONESE MALES. MEAN WEIGHTS AT DIFFERENT AGES.

Age Group in Years.	Number in Group.	Mean Weight in Lbs.	Standard deviation of the Weight.
16	373	97.08	9.28
17	397	101.63	9.70
18	294	105.92	9.44
19	481	110.19	9.58
20	276	110.76	11.32
21	472	109.34	10.63
22	388	110.82	10.12
23	422	110.46	11.22
24	530	110.14	11.26
25-29	2549	110.47	11.30
30-34	1817	110.32	11.14
35-39	1322	110.46	11.30
40-44	677	110.27	11.74
45-49	542	109.67	12.64
50-54	511	106.28	11.66
55-59	424	105.10	11.52
60 +	372	105.22	11.80
All ages ...	11,847	108.97	12.10

TABLE III.  
CANTONESE MALES. MEAN WEIGHTS AT DIFFERENT HEIGHTS.

Height in Inches.	Number in Group.	Mean Weight in Lbs.	Standard deviation of the Weight.
Under 59	73	89.87	8.18
59-	187	94.74	7.90
60-	522	97.61	8.72
61-	939	99.87	8.42
62-	1514	103.60	9.08
63-	1939	106.40	9.34
64-	2182	109.19	9.75
65-	1865	112.31	9.98
66-	1260	114.48	10.30
67-	719	117.67	11.10
68-	398	120.65	10.40
69-	159	123.39	11.34
70 +	90	128.65	11.14
Total ...	11,847		

TABLE IV.

CANTONESE MALES. MEAN HEIGHTS FOR DIFFERENT WEIGHTS.

Weight in Lbs	Number in Group.	Mean Height in Inches.	Standard deviation of the height.
Under 79.5	26	59.99	1.73
-83.5	75	61.28	1.71
-87.5	165	61.59	1.88
-91.5	358	61.87	1.82
-95.5	699	62.43	1.86
-99.5	1285	62.92	1.83
-103.5	1467	63.49	1.80
-107.5	1584	63.88	1.82
-111.5	1619	64.52	1.87
-115.5	1450	64.78	1.80
-119.5	1030	65.15	1.79
-123.5	808	65.68	1.88
-127.5	510	65.90	1.92
-131.5	339	66.45	1.89
-135.5	223	66.70	1.92
-139.5	87	66.89	1.78
-143.5	64	67.23	2.11
143.5 and over	56	67.68	1.96
Total	11,847		

order to render these statistics comparable to similar ones from Europe and America, I have deducted one year from the Chinese ages as stated on the prisoners' history sheets.

The coefficient of correlation between height and weight is 0.551. The coefficient of regression of weight in terms of height is 2.974, *i.e.*, for every inch increase in height there is an increase of 2.974 lb. in weight. The accompanying graph shows the observed and calculated regression lines for height and weight.

I have not met with any papers dealing with heights and weights in Cantonese adults, but there are two concerned with school boys which give details up to the ages of 21 and 22 years, so I have extracted the relevant material for comparison with the younger age groups of my series. They are very small series, but are all that are available. The two papers, one by KEYS and CADBURY (1926) and the other by STEVENSON (1925) are concerned with boys of the middle classes in Canton, attending school under good hygienic conditions.

It will be seen that at corresponding ages they are 6 to 9 lb. heavier, and from  $\frac{1}{2}$  to  $1\frac{1}{2}$  inches taller than the Cantonese of the lower classes of Hongkong.

This difference is a measure of the degree of under-nourishment among the latter, and indeed among the lower classes generally of South China. In STEVENSON's paper the values are expressed in cm. and kg., which I have converted to inches and lbs.

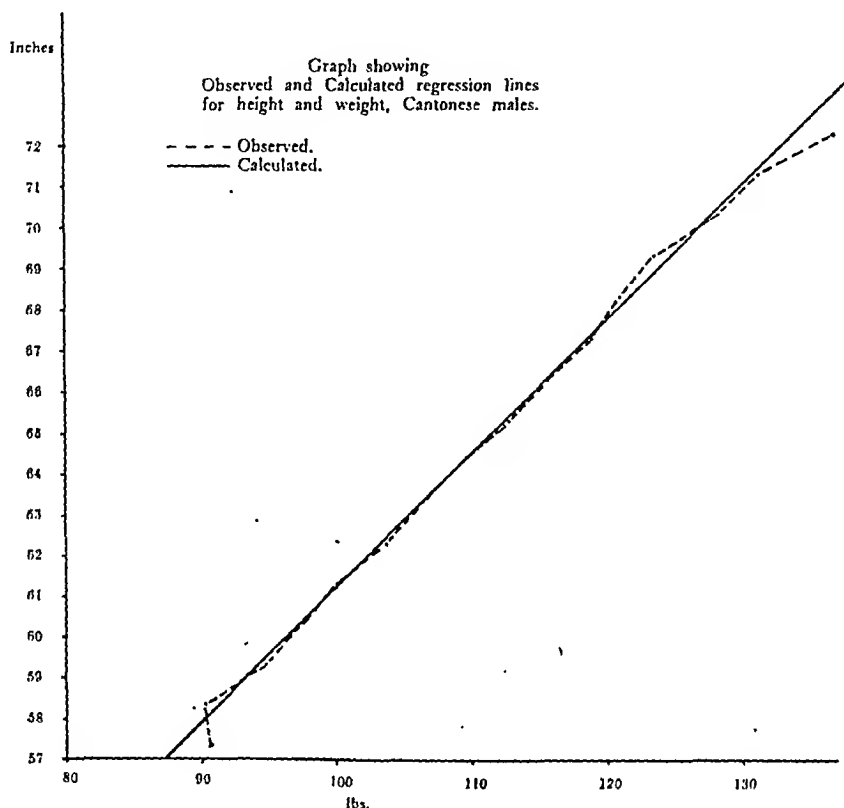


TABLE V.

HEIGHTS OF CANTONESE YOUNG MEN, ACCORDING TO VARIOUS AUTHORITIES.

Age in Years.	KEYS & CADBURY			STEVENSON.		UTTLEY.		
	Number in Group	Weight in Lbs.	S.D. of Weight.	Number in Group.	Weight in Lb.	Number in Group.	Weight in Lbs.	S.D. of Weight.
16	91	63.79	2.04	103	64.57	373	62.25	1.34
17	91	64.53	1.96	102	65.00	397	62.89	2.01
18	81	65.02	1.96	94	65.04	294	63.55	2.14
19	56	65.26	2.06	83	65.16	481	63.87	2.17
20	56	65.26	1.64	54	65.00	276	64.54	2.15
21	—	—	—	28	65.12	472	64.27	2.20
22	—	—	—	47	64.80	388	64.48	2.10
23	—	—	—	—	—	422	64.56	2.16

TABLE VI.

WEIGHTS OF YOUNG CANTONESE MEN, ACCORDING TO VARIOUS AUTHORITIES.

KEYS & CADBURY.				STEVENSON.		UTTLEY.		
Age in Years.	Number in Group.	Weight in Lbs.	S.D. of Weight.	Number in Group.	Weight in Lbs.	Number in group.	Weight in Lbs.	S.D. of Weight.
16	91	106.74	12.94	103	103.86	373	97.08	9.28
17	91	110.86	11.22	102	107.60	397	101.63	9.70
18	81	114.17	10.74	94	112.23	294	105.92	9.44
19	56	116.34	9.82	83	114.66	481	110.19	9.58
20	56	116.84	10.61	54	110.69	276	110.76	11.32
21	—	—	—	28	111.57	472	109.34	10.62
22	—	—	—	47	109.81	388	110.82	10.12
23	—	—	—	—	—	422	110.46	11.22

## SUMMARY.

An investigation into the heights and weights of over 11,800 healthy adult Cantonese males admitted to the Hongkong prison showed that the mean height was 64.23 inches (163.14 cm.), with a standard deviation of 2.24. The mean weight was 108.98 lb. (49.43 kg.), with a standard deviation of 12.10.

The coefficient of correlation between height and weight was 0.551.

For every increase of 1 inch in height there was an increase of 2.974 lb. in weight.

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## CHOLERA AND ANURIA

BY

J. WALKER TOMB, *O.B.E.*, M.D., D.P.H.

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### ANURIA.

The four cardinal symptoms of cholera consist of purging, vomiting, muscular cramps and suppression of urine.

According to WINTON (1937), when the systolic blood-pressure falls to 75 m.m. Hg., the secretion of urine ceases.

ROGERS (1911) writes: "The most justly dreaded late complication of cholera is continued suppression of urine after reaction from collapse has taken place, as, unless it be of strictly limited duration, it leads to the supervention of uraemia combined with toxæmia." (The term "uraemia" is used by ROGERS and his followers to connote anuria with urea-retention and is without its proper clinical significance.)

HALLIBURTON and McDOWALL (1937) write: "Uraemia is the term given to the rapidly fatal state, commonly associated with unconsciousness, which results from severe disease of the kidney. The term was originally applied on the erroneous supposition that it is urea or some antecedent of urea which acts as the poison. There is no doubt that the poison is not any constituent of normal urine. If the kidneys of an animal are extirpated the animal dies in a few days, but there are no uraemic convulsions. In man also if the kidneys are healthy, or approximately so, and suppression of urine occurs



from simultaneous blocking of both renal arteries by clot, or of both ureters by stones, again uraemia does not follow. On the other hand, uraemia may occur even while a patient with diseased kidneys is passing a considerable amount of urine. What the poison is that is responsible for the convulsions and coma is not known. It is doubtless some abnormal katabolic product, but whether this is produced by the kidney cells or in some other part of the body is also unknown."

LANGDON-BROWN and EVANS (1937) write: "Uraemia is the name which was given in the past to the toxic state which complicates or terminates severe kidney disease and in which urea-retention occurs."

More recently (they add) the name "non-renal uraemia" has been given to the high grade of urea-retention which may develop as a result of non-renal factors. Amongst the most important of these factors are vomiting and diarrhoea, both of which cause urea-retention through loss of body fluids and the accompanying loss of chlorides.

The symptoms of "choleraic uraemia," *i.e.*, anuria, are thus described by ROGERS (*loc. cit.*): "The pulse remains above the normal rate and tends to increase instead of falling. More important is a quickening and deepening of the respirations . . . which should at once put the physician on his guard and lead to the adoption of vigorous measures to try to avert the threatening calamity. Vomiting may recur, but it is not usually a marked symptom. . . .

"The skin is dry, and sweating may sometimes be difficult to produce in unfavourable cases. Diarrhoea may persist. . . .

"If the action of the kidneys cannot be restored the respirations become more and more laboured, restlessness ensues, and after a struggle for breath lasting for several days, gradual clouding of the intellect—deepening into coma, or cardiac failure—ends the scene."

OSLER (1938) states: "Weakness, vertigo, nausea, dullness and drowsiness passing into coma, are the usual features of anuria. . . . Vomiting is fairly common."

The experimental administration of massive doses of urea causes a similar group of symptoms\* (LANGDON-BROWN and EVANS, *loc. cit.*).

The most prominent clinical symptoms of acute renal uraemia are severe headaches and mental disturbances, sudden amaurosis, convulsions and coma: a symptom-complex which is never met with in cholera.

#### MECHANISM OF URINARY SECRETION.

Regarding the mechanism of urinary secretion HALLIBURTON and McDOWALL (1937) state: "One fluid, the arterial blood, enters the kidney: two fluids, the venous blood and the urine leave it. Both of these fluids are different in composition from arterial blood. . . .

\* Headache, giddiness, apathy, drowsiness, weakness, nausea and diarrhoea.

"We know that it is not possible to convert any fluid into two others, each of different composition from itself, without an expenditure of energy which must come from somewhere outside the fluids themselves. In the kidneys, as in other secreting glands, this energy comes from the cells of the organ and the pressure of the arterial blood. The secretion of urine is therefore the result of work done by the kidney. The quantity of work done may be measured within certain limits and the energy transformed by the kidneys may be estimated [in several ways]. . . .

"Estimations have been made of the amount of oxygen used by the kidney in secreting urines of known concentration. This oxygen may be taken as a measure of the amount of energy used by the organ. . . .

"The practical importance of these considerations . . . lies in the fact that the expenditure of energy involves combustion, and combustion demands oxygen. For this reason an efficient supply of oxygen is essential to . . . healthy kidney [function]. . . .

Regarding the secretory power of the glandular epithelium of the kidney tubules, HALLIBURTON and McDOWALL (*loc. cit.*) write: "In frogs the glomeruli can be cut out of action by ligaturing the renal arteries. The kidney is then supplied by the renal-portal vein, a vessel which goes to the tubules only. If urea is then injected under the skin, secretion of urine occurs which, though scanty in amount, is peculiarly rich in urea. Urea therefore in the frog is secreted by the epithelium of the tubules. In order to obtain this result, the kidney must receive sufficient oxygen for the maintenance of the functional activity of its cells. As the arterial supply is cut off by ligature of the renal arteries, this must be accomplished . . . by keeping the frog in an atmosphere of pure oxygen."

#### POSTMORTEM APPEARANCE OF KIDNEY IN CHOLERA.

With reference to the postmortem appearance of the kidney in cholera CHATTERJEE (1941) writes: "From the postmortem records of fifty-eight cases of cholera in the Carmichael Medical College Hospital [Calcutta], the gross appearance of the kidneys to the naked eye showed a marked congestion in 5 per cent., moderate congestion in 36 per cent., and a practically normal structure in 59 per cent." . . .

"Acute inflammatory changes are as a rule absent in the uraemic as well as in the non-uraemic kidney of cholera."

The histological changes found in the kidney in so-called "uraemic" cases of cholera are thus described by CHATTERJEE (*loc. cit.*): "The Malpighian corpuscles show great thickening and splitting of the basement membrane as well as non-inflammatory dilation and congestion of the capillaries of the glomerular tuft. As a result, Bowman's capsule is seen to be more or less completely filled up. Similar swelling of the basement membrane of the

tubules and dilatation of the capillaries of the medulla are also observed" . . . "Sometimes . . . the cells of the [convoluted] tubules might show degenerative changes" [30% in one series of cases—the proportion, doubtless, depending inversely on the resistance of the epithelial cells of the tubules to tissue-asphyxiation]. . . . "The changes consist of a fatty degeneration of the [epithelial] cells of the convoluted tubules . . . and also of a degenerative swelling of the cells, so that the lumen of the tubule is obliterated."

#### COLLAPSE.

Since collapse of the circulation in consequence of the loss of body fluid is the outstanding symptom of fully-developed cholera, it will be profitable to consider the pathology of collapse.

BAYLISS (1919) writes: "The second pathological state induced by prolonged low blood pressure in the capillaries is an increase in the permeability of the blood-vessels to colloids. Their normal state is one of impermeability to colloids, so that a solution of a colloid of sufficient osmotic pressure does not leave the circulation. If this property of the capillaries is lost, there is no force to retain fluid and both gum-saline and blood plasma escape into the tissues. The clinical index that such a state has been reached or is coming on is a progressive concentration of the blood as regards corpuscles. As long as the blood vessels maintain their normal state, the effect of a fall in blood pressure is to attract fluid from the tissues. This is because the osmotic pressure of the colloids remains at its normal height, while the filtration is reduced owing to the low arterial pressure. The result is a dilution of the blood, which is a favourable sign. . . .

"If the increased permeability has not reached too high a value or not been present for too long a time, recovery is possible. . . . It is evident, then, that the state is capable of return to normal, if not too serious. The renewed supply of oxygen restores the necessary impermeability to colloids."

Regarding the collapse of the circulation, as observed in experimental animals, BAYLISS (*loc. cit.*) states that if treated shortly after onset recovery takes place, but if left for two hours or more . . . recovery is impossible.

O'SHAUGHNESSY and SLOME (1935) also state: "There is no satisfactory treatment of shock that has persisted for several hours. The subject of severe trauma who does not show some signs of recovery under established modes of treatment within two to three hours of his injury is almost inevitably doomed."

#### CAUSE OF FAILURE OF SALINE TRANSFUSIONS.

The frequent failure of saline transfusions to restore and maintain the circulation in cholera, as well as to re-establish the secretion of urine, in cases

where the circulation has been successfully restored, is thus seen to be due to irreversible change in the capillary endothelium, as well as in the epithelial cells of the kidney tubules, from lack of oxygen.

### RENAL FAILURE AFTER TRAUMATIC SHOCK.

In the condition known as "crush" injury, in which renal failure (anuria) is found to follow upon successful treatment of collapse of the circulation from traumatic shock after prolonged burial under débris consequent upon air raids, BEALL *et al.* (1941) report that the renal lesion in these cases "consists structurally of severe degenerative changes in the proximal convoluted tubules, and in the more distal parts of the nephron, brown pigmented casts. The matrix of the casts is thought to be composed of desquamated epithelial cells."

RENNIE, J. B. (1941), calls attention to two cases of renal lesions from traumatic shock described by HUSFELDT and BJERLING (1937). Both patients sustained fracture of the pelvis and damage to soft tissues. Shock developed in both, yielding to transfusion of citrated blood. Death occurred on the eighth day with a rising blood urea, hypertension, oedema (in one case), and general signs of "uraemia." The urine was very scanty, of low specific gravity, and contained casts and a few erythrocytes. Renal function tests gave very low results. At necropsy no injury to kidneys or urinary tract was found. Microscopically the chief lesions were emptiness of the glomerular capillaries and degeneration of the epithelium of the tubules (in some of which granular coloured pigment was present).

It would therefore appear that the epithelial cells of the convoluted tubules are even more sensitive to want of oxygen than the endothelium of the blood capillaries itself.

### TREATMENT OF COLLAPSE.

Since the effects produced by collapse of the circulation from any cause, including cholera, are directly referable to tissue-asphyxiation of the body-cells from want of oxygen (anoxia) I suggest that oxygen be administered where available (by B.L.B. mask) in all cases of collapse in cholera, after preliminary restoration of the circulation by intravenous salines or by salines with plasma.

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## FILARIASIS OF THE BREAST: A MAMMOGRAPHIC STUDY

BY

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Extensive mammographic studies by HICKEN and co-workers (1937) and by GERSHON-COHEN and COLCHER (1937) show that lipoma, fibro-adenoma, simple retention cysts, cystic degeneration of the lactiferous ducts and carcinoma can all be correctly visualized and diagnosed by this method.

Filariasis of the female breast occurs fairly frequently in the heavily filaria-infested Pacific islands. Diagnosis of the condition rests upon the locality where the patient lives, history of attacks of "fever" with pain and swelling of the breast, the presence of microfilariae in the blood and positive serological or intradermal tests. However, microfilariae are frequently absent from the blood, and material for the performance of auxiliary tests may not be available; moreover, changes in the breast due to filariasis may not occur in young women but in older ones, where the possibility of neoplasms must be considered. In such doubtful cases the use of mammography may occasionally save the patient an unnecessary operation.

The case history given below illustrates such a type of case, but here the presence of microfilariae minimised the value of the radiological findings, although they served to exclude the possibility of neoplasms as a complicating factor.

### CASE HISTORY.

E., female Nauruan, aged approximately 52. Seen on 7th November, 1940, when she complained of swelling and pain in the right breast. Many previous attacks of a similar nature had occurred and on one occasion the breast had been incised without, however, relieving or curing the condition. The right breast was found to be enlarged, reddened and tender. The nipple was enlarged and hard. One area of the overlying skin was tense and displayed marked *peau d'orange*. Palpation, which produced pain, revealed a mass in the external upper quadrant. One enlarged, tender lymphatic gland was palpated just under the lateral edge of the right pectoralis major muscle. No enlargement of the axillary lymphatics was detected. A pigmented, keloidized scar, the evidence of the incision mentioned in the patient's history, was seen at the upper and outer quadrant. The left breast was normal. Microfilariae were found in the night blood. The acute attack was successfully treated with

\* My thanks are due to Dr. B. H. QUIN, Senior Medical Officer, Administration of Nauru, for permission to investigate the case, and to Mr. W. SHUGG, Medical Assistant, Nauru, who is responsible for the photographs.

M. & B. 693, as has already been reported (EARLE, 1941). Following subsidence of the acute condition, the breast was investigated radiologically.

### *Radiological Investigation.*

In all the radiographs illustrated the following technical factors were used : Distance, 90 cm. ; Kv.P., 57 ; Milliamperage, 15 ; time of exposure, 3 seconds.

To get the breast into good position, a home-made cradle, modified from that described by GERSHON-COHEN (1937) was used.

Ordinary radiographs of the affected breast, together with that of the opposite side as a control, are illustrated in Figs. 1 and 2 respectively. Little difference is seen between healthy and diseased breasts, except that an increase in fibrous tissue occurs in the diseased organ. There is, however, in the affected breast nothing to suggest that the mass felt was of neoplastic origin ; neoplasms, when viewed by this method, may show irregular spreading margins with delicate radiating striations arising from the periphery (malignant) or smooth, clear-cut margins (benign).

Later still, when the inflammatory process had further diminished, two more radiographic examinations were made, one "straight" and the other after injection of lipiodol into the lumina of the lactiferous ducts (Figs. 3 and 4).

In Fig. 3, apart from an increased amount of fibrous tissue, there is nothing of diagnostic significance. Fig. 4 shows a multiparous breast which, however, is not quite like that of a young resting multiparous breast, since the ducts are less numerous. This can be explained by (1) post-climacteric atrophy, and (2) the increased fibrous tissue, following repeated inflammatory attacks, leading to obliteration of many of the ducts. However, the ducts which remain patent ramify in the matrix in a uniform manner and show no evidence of displacement or destruction. (Displacement and filling defects are associated with papilloma : adenocarcinoma produces a compressed, moth-eaten appearance in the ducts, which in some cases are completely destroyed.)

### SUMMARY.

1. The radiographic investigation of a case of filariasis of the breast is described.
2. The use of the method as a differential diagnostic measure in certain obscure cases is indicated.

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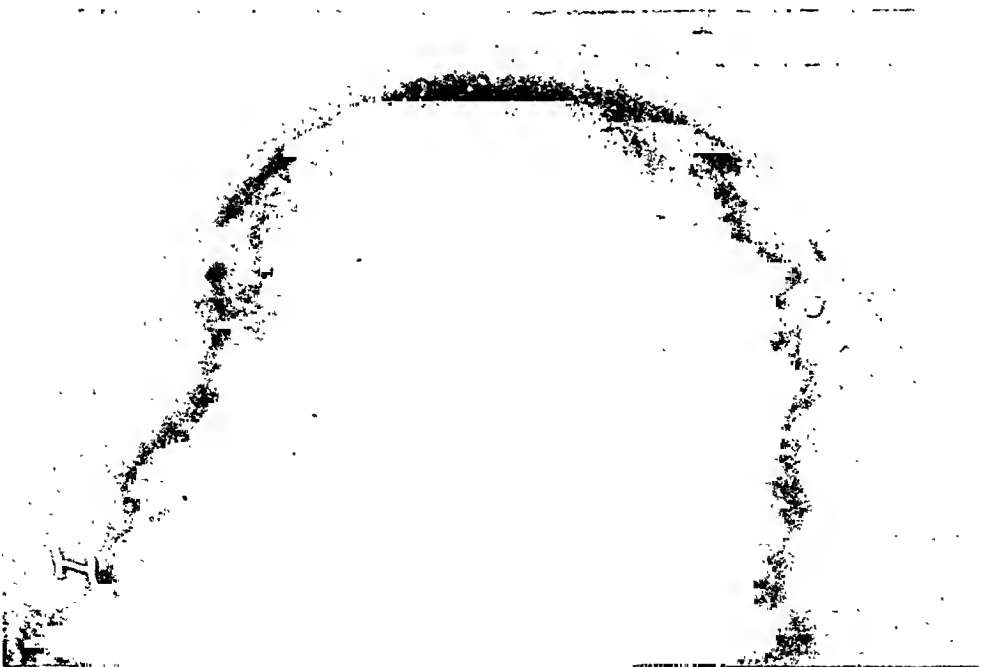


FIG. 1.—Right breast. Note increased density, as compared with normal side, Fig. 2. (7.11.40).

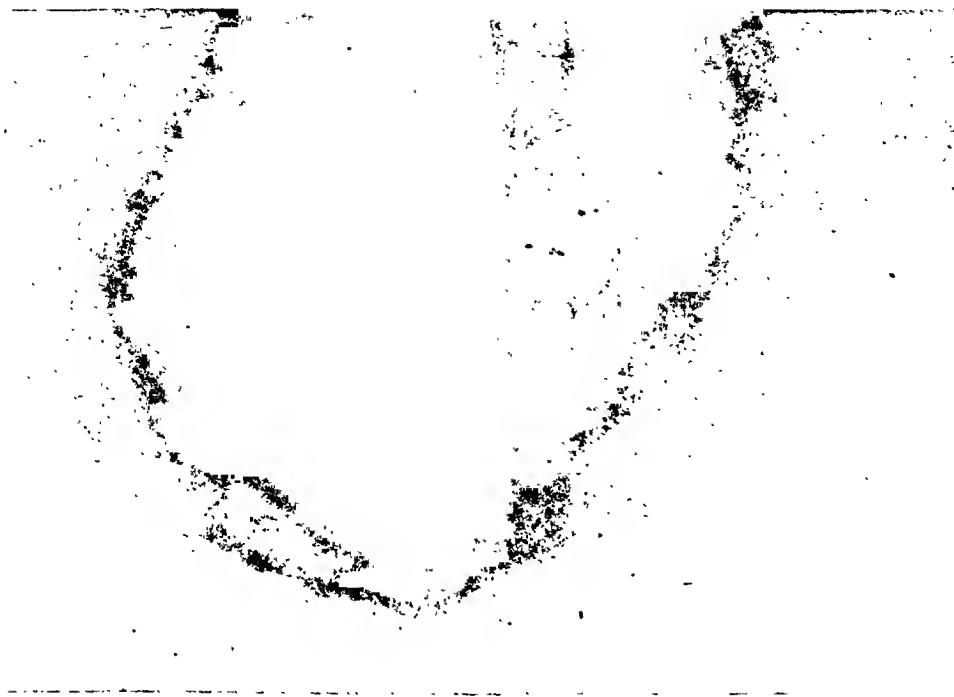


FIG. 2.—Left breast. Normal. (7.11.40).





FIG. 3.—Right breast. (19.12.40).



FIG. 4.—Right breast, injected with lipiodol, showing lactiferous ducts (19.12.40).

## CORRESPONDENCE.

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### ZOOLOGICAL NOMENCLATURE AS APPLIED TO MEDICAL ZOOLOGY, PARASITOLOGY AND BACTERIOLOGY.

WITH SPECIAL REFERENCE TO THE STATUS OF THE PACIFIC FILARIA.

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*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In your issue of 29th November, 1941,\* my friend and colleague, Dr. G. CARMICHAEL LOW, quite justly takes me to task for overstepping the bounds of strict zoological propriety in my suggestions regarding the nomenclature of the Pacific filaria. He would keep to the strait and narrow path, and in so doing has sought to make "the punishment fit the crime." In making this claim I was fully aware of the zoological indiscretion I was committing, but in spite of his considered judgment on this matter, I still remain of the opinion that strict application of the International Rules of Zoological Nomenclature to medical science cannot always be upheld and, indeed, in many instances, as I shall point out, they have already been grossly infringed. In Parasitology it is becoming increasingly clear that strict adherence to morphology as a criterion of specificity is not maintained so that the present rules merely tend to impede progress.

Brief consideration of our subject reveals several instances where biological behaviour in its bewildering many-sided phases has determined the identity of a lowly organism.

The tenets of zoological nomenclature regulate our needs when applied to the higher forms of life, and I quite realise that, in this sphere, any departure from the law of the Medes and Persians is bound to lead to confusion, but in Parasitology, mainly because of the confined environment in which these creatures are constrained to live, biological features tend to become of paramount importance, and indeed I go so far as to say that this applies to the whole of Medical Zoology. In Medical Entomology, for instance, there are admittedly several biological races of *Anopheles maculipennis*, of which the adult forms are identical, but which lay different eggs, have different habitats, different feeding habits, but which from the aspect of malariology are perfectly distinct, so distinct that measures devised against one are ineffective against another. Their separation has signalized an important step in the epidemiology of malaria. It is just as vital for the sanitarian to recognise this fact as it is to admit the specific distinctions in the genus *Culex* or *Aedes*.

\* LOW, G. CARMICHAEL. (1941). The Nomenclature of the Pacific Filaria. *Trans. R. Soc. Trop. med.*, 35, 197.

In Protozoology the case of the trypanosomes can be cited. On what valid grounds can *Trypanosoma rhodesiense* be separated as a species distinct from *T. gambiense*? I do not assert that it is not, but its status appears to be a counterpart of the Pacific filaria. One cannot claim that in this trypanosome specific rank can be maintained on the basis of morphology or, as was formerly held, on changes in the position of the nucleus which may be observed in another host (rat), but solely on the facts that it is transmitted by a separate species of *Glossina*, has a different geographical distribution and possibly produces more virulent pathological changes. Nor can systematists pretend that other mammalian trypanosomes, such as *T. brucei* or *T. evansi*, can be separated with certainty on morphological grounds. But who is there who would deny that they represent specifically distinct species? Then there are the amoebae of monkey, man, and rat which are apparently interchangeable. Moreover the type *Entamoeba histolytica* of Schaudinn, if the rules were strictly enforced, should be properly known as the *Amoeba coli* of Lösch. This surely is a question of priority which has never been adhered to. In leishmaniasis also it is clear that zoological nomenclature breaks down entirely. There can be no justification for such specific terms as *Leishmania infantum*, *L. americana* or *L. tropica* when these parasites are indeed morphologically identical with *L. donovani*; but medical opinion demands, in view of the totally dissimilar conditions produced, that there must be some specific determinative terminology. The same considerations apply to the Spirochaete group, *Sp. duttoni*, *Sp. hispanica*, etc., which should strictly become synonymous with *Sp. recurrentis*.

But when we come to the realm of the infinitely small and to bacteriology it is quite evident that our Zoological Nomenclature breaks down entirely. Mere superficial contemplation of the varied organisms of the enteric and dysentery groups is enough, not to mention the many apparently authentic specific variations of the pathogenic streptococci. Therefore, if mere morphology were maintained in this sphere, the whole of our modern bacteriological nomenclature would be brought to nought. I maintain that these are valid arguments and germane to the subject under discussion.

It is admitted that the Pacific filaria has certain distinctive biological peculiarities, a defined geographical distribution and a distinctive intermediary host and therefore may be regarded as distinct from *W. bancrofti* as *T. rhodesiense* is from *T. gambiense*. What is "sauce for the goose is sauce for the gander," so that from the parasitological and pathological aspect advance in knowledge can only be made by the recognition of the justice of this view. I would submit that the strait and narrow path cannot be applied to Medical Zoology. Whilst admitting that we should strive for something better—some compromise which will legislate for these modern difficulties and which will have regard for the fact that "nature" moves in a mysterious way her wonders to perform.

There is another serious aspect of this affair which is disconcerting to the student and practitioner alike and that is the almost inextricable medley of names with which Tropical Medicine has become encumbered, mainly by the efforts of systematic zoologists. I feel certain that this is an incubus which causes many to steer clear of this absorbingly interesting branch of medicine. Since I have known her *Aedes aegypti*, like some favourite film star, has changed her name no less than four times, and one wonders whether the authorities are even now entirely satisfied. I need hardly mention the case of *Bilharzia*, *Schistosoma* or *Schistosomum*, for it is not yet settled to which sex this sexually-minded trematode belongs.

I would issue a plea for the inception of a new International Standard of Nomenclature as applied to Medical Zoology and Parasitology. This is a burning question and one which will have to be squarely faced sooner or later for, as systematic zoology is not concerned with biological surroundings, pathological and biochemical effects, why should zoological rules based solely on morphology determine the ordered progress of Medical Science as regards nomenclature?

We are therefore faced with the choice of two courses. Either we must stick to the strict application of the International Rules of Zoological Nomenclature, as Dr. CARMICHAEL LOW would have us do, or we must adopt a new system on the lines I have indicated. In either case some regrouping and renaming will have to be undertaken to bring Medical Zoology into line. I would favour the alternative which would entail the setting up of a new standard of scientific nomenclature as applied to medicine.

I am, etc.,

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London.

PHILIP MANSON-BAHR.

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### TEMPERATURE OF MAMMALS.

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To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

SIR,

In No. 1, Vol. XXXV, of the TRANSACTIONS there is an article by a colleague of mine, Mr. F. L. VANDERPLANK\*, published while I was away from the department. In this article on the relation between the virulence of *T. rhodesiense* towards rats and the normal blood temperature of its previous mammalian host, there is a basic error that can only be recognised by those (mainly veterinarians) who have had long experience of temperaturing animals in tropical countries. The error is the implication that there is such a thing as a normal

\* A note on the relation between the virulence of *Trypanosoma rhodesiense* towards rats and the normal blood temperature of its previous mammalian host.

temperature for every species of mammal, even though the great majority differ from the very few—*e.g.*, man and the horse—by not sweating freely. Those animals are not homothermic, and their temperature varies greatly throughout the hours of a hot day. So well known is this to veterinarians that in tropical climates the interpretation of temperatures taken at any hours except those of early morning is considered a matter of difficulty. REAGAN and RICHARDSON in California kept cows in a large room in which the temperature could be varied from 40° to 100° F., while the relative humidity was maintained at 60 per cent. and the air movement at 60 cubic feet per minute. It was found that as the temperature increased over this range the pulse rate of the cows decreased from 72 to 57 beats per minute. At the same time the respiration rate closely followed Van't Hoff's law for chemical reactions and was approximately doubled for every increase of 18° F., rising from 12 to 124 respirations per minute between 40° and 100° F. The rectal temperature of the cows remained almost constant at 101-101·3° F. when the room temperature varied between 40° and 70° F., but above that point the rectal temperature steadily increased until it attained 105·1° F. at an external temperature of 100° F. In the Mpwapwa veterinary laboratory all routine temperaturing of experimental animals is done before breakfast, and comparative figures for sheep and goats temperatured daily for long periods are available. From these I should say that the expected early morning temperature of a healthy adult sheep is about 102° and that of a goat 101·5° F., but in either species the actual reading may be anything between 100° and 103° F. Young animals show greater variations in temperature than adults of the same species, and excitement or struggling will quickly send up the temperature of an animal of any age, even on a cool morning.

2. From these remarks it will be seen that many more than the number of observations made by Mr. VANDERPLANK are necessary before comparative figures like those in Column 3 of Table II of his article can be accepted.

3. This criticism does not detract from the interest of the observations in support of the view that virulence of trypanosomes towards rats may be modified differently by different species of mammalian hosts, but it does question the soundness of the hypothesis that the modification is a function of the "normal blood temperature" of the host.

I am, Sir, etc.,

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[Seconded as *Food (Animal Products) Controller.*]

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# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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## COMMUNICATIONS.\*

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### TROPICAL ULCER IN TRINIDAD.†

BY

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*(Late Senior Resident Surgeon, General Hospital, Barbados, British West Indies; Medical Officer, United British Oilfields of Trinidad, Ltd., Trinidad, British West Indies).*

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The crippling condition of tropical ulcer is widespread throughout the Colony of Trinidad and responsible for much loss of time in local industry. In this paper the clinical aspects of the disease as it occurs among oilfield workers and their families in the southern part of the island are described. Some facts relating to the disease in the colony as a whole are also given and, where of interest, comparisons are made with the findings of other observers in other countries.

#### ETIOLOGY.

##### *Distribution.*

1,011 consecutive work-applicants, supposedly healthy, were inspected for active ulcers or scars indicating past infection. The number of active ulcers was not expected to be large since it was announced that those with leg ulcers would be rejected: this is necessary since the condition is a severe handicap to the worker's efficiency and, if undetected at the initial medical examination, often leads to spurious claims for disability compensation. In this inspection,

\* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

† Abstracted from a Dissertation, University of Cambridge.

therefore, the value lies in the figures obtained for the scars of past infection. The findings are shown in Table I.

TABLE I.

Race	Number of men examined.	Ulcer scars.	Percentage with scars.	Active ulcers.
East Indian ...	168	38	22.63	Nil
Negro ...	320	153	18.60	3
Mixed creole ...	16	2	12.50	Nil
Chinese ...	7	Nil	—	Nil

It is seen that the incidence of past infection is higher in East Indians than in negroes. The numbers of mixed creoles and Chinese were too small to admit of profitable deductions.

Of interest are the figures published by the Director of Medical Services of Trinidad and Tobago, relating to cases of "ulcer" admitted as in-patients to the Colonial and District Hospitals. It is to be regretted that such a broad classification has been adopted, but it may be taken that the vast majority of these ulcers are of the tropical variety. Other ulcers (*e.g.*, syphilitic, diabetic) have been included under their respective causative diseases.

TABLE II.

Year.	Total hospital admissions (All diseases).	Admissions for "ulcer."	Deaths from ulcer
1937	21,640	354	1
1938	21,718	344	4

From this table it is seen that out of the total number of hospital admissions 1.63 per cent. were for "ulcer."

Apart from those cases admitted as in-patients there is a huge, but unrecorded, number of patients who receive out-patient treatment only. Furthermore, there remain those who treat themselves with herbs and patent nostrums or are in the hands of obeah men and quacks.

#### *Dietetic Factors.*

The diet of the Trinidad negro labourer is a notoriously poor one, being deficient in animal protein, animal fat, calcium, phosphorus and vitamins A, B<sub>1</sub>, B<sub>2</sub>, C and D and containing an excessive amount of carbohydrate (EARLE, 1941). East Indians have a yet poorer diet composed almost entirely of carbohydrates and practically devoid of first-class protein, animal fat, minerals

and vitamins B<sub>2</sub> complex, C and D: small amounts of vitamins A and B<sub>1</sub> are included. It is likely that the higher incidence of tropical ulcer among East Indians than among negroes is dependant on the poorer diet of the former race.

Dietetic factors studied by other observers are of interest. Thus CLEMENTS (1936), in a survey of tropical ulcer occurring in sago-eating tribes in the island of Manus (New Guinea), found that the disease is associated with a dietary in which there is a partial or complete deficiency of vitamin B<sub>2</sub> complex, abundance of carbohydrates and small amounts of protein and fat: there was a high calcium content in the diet.

ORR and GILKS (1931), comparing two East African tribes—the Akikuyu and Masai—found the incidence percentage of tropical ulcer to be 1.60 in the former and 0.16 in the latter. This large difference was explained by the following dietetic defects in the Akikuyu diet—(i) low protein and fat intake; (ii) low biological value of the proteins taken; (iii) low vitamin content (especially vitamin B<sub>2</sub> complex); (iv) low calcium content.

In Northern Nigeria, McCULLOCH (1928) and BURNIE (1931) showed that tropical ulcer occurred principally among natives who subsisted on a diet consisting largely of millet and guinea corn. These foodstuffs have a low content of vitamins A, B<sub>2</sub> complex and C.

Deficiency of the vitamin B<sub>2</sub> complex is a common factor in all the above sets of findings and it is likely that this deficiency is a main predisposing cause of tropical ulcer. Other dietetic deficiencies also probably play a minor rôle in producing the disease.

### *Incidence.*

*Age Incidence.*—In forty-six consecutive cases of tropical ulcer the age distribution and incidence was as follows:—

TABLE III.

Decades	1st (1-10 years)	2nd (11-20 years)	3rd (21-30 years)	4th (31-40 years)	5th (41-50 years)
Cases of tropical ulcer	10	12	17	5	2

The lowest age was 5 and the highest was 43.

Children under the age of 5 are rarely affected, a fact that has also been noted by AROSOLIDES (1922). There appears to be a degree of immunity enjoyed by the very aged, though an ulcer developing in a senile West Indian often proves to be fatal; a mishap that I have seen occur many times in Barbados.

The maximum incidence of the disease occurs in third decade. This is



the age of the majority of workers and exposure to traumata is therefore more frequent during this period of life:

*Sex Incidence.*—In forty-six consecutive cases of the disease 31 (67·38 per cent.) were males and 15 (32·62 per cent.) were females. This is readily explicable since men, working in the oilfields, are more frequently exposed to the type of injury which is the starting-point of tropical ulcer.

*Racial Incidence.*—In the oilfields, tropical ulcer is seen more frequently among negroes than among any of the other races. This is due to the fact that this race predominates in the Trinidad oil industry. Heavy, unskilled labour with the attendant bruises and lacerations is for the most part performed by the negro race. The children of negroes and East Indians, who run bare-foot, provide the vast majority of juvenile cases; those of white and mixed creoles, Chinese, Europeans and white Americans are relatively free of the disease.

Table IV shows the racial distribution in one of my series of cases.

TABLE IV.

Race.	Percentage incidence.
Negro ...	78·2
European ...	8·69
East Indian ...	6·52
Chinese ...	4·39
Mixed creole ...	2·2

It must be remembered that the figures in Table IV deal with oil workers only. Table I indicates that when large numbers of men are examined, scars indicating past ulceration are more frequent among East Indians.

#### *Seasonal Variation.*

In Trinidad season does not appear to exercise any great influence on the incidence of tropical ulcer although the months February to August (inclusive) were those in which the maximum number of cases were seen. This period bears a very slight relationship to lowest maximum atmospheric temperature, low rainfall and low relative humidity. Whether these meteorological conditions have any bearing on the vitality of the human subject or on the vitamin-content of foodstuffs has not been determined.

Conflicting findings have been made by other observers. Thus CORPUS (1924) states that in the Philippine Islands the highest incidence is during the hot and damp months (August to October); the disease decreasing during the dry season. In Nigeria the increase of new cases admitted to hospital coincides with the end of the rainy season (BURNIE, 1931), but SMITH (1914) in the same country found the highest incidence in his series of cases to be related

to the months of maximum rainfall. EGGERS (1915) in China noted that the maximum number of cases occurred during early and late summer. ONORATO (1929) states that the highest incidence occurs during the periods of drought and heat. In Manus (New Guinea), CLEMENTS (1936) found the highest number of hospital admissions with this condition to occur during the monsoon season and the three months following (January-June).

In the table which follows, the meteorological readings were taken at the St. Clair Experimental Station, Port-of-Spain, longitude 61° 31' W., latitude 10° 40' N.; the barometer being 72 feet above mean sea level.

TABLE V.

Month.	Temperature.				Rainfall.		Tropical ulcer.
	Maximum.	Minimum.	Range.	Mean.	Amount in inches.	Relative humidity (mean %)	% of total Cases
January ...	92.2	68.7	23.5	80.1	3.56	84.0	7.37
February ...	92.5	67.3	24.9	78.8	1.94	78.3	13.47
March ...	93.5	67.7	25.9	80.6	2.33	77.0	9.21
April ...	92.1	68.6	25.5	80.3	4.52	79.2	8.60
May ...	92.7	70.0	22.0	84.2	3.49	77.5	10.44
June ...	92.7	71.3	21.4	81.9	5.10	79.3	9.82
July ...	91.6	68.8	22.8	80.7	8.37	84.0	10.44
August ...	91.9	70.2	21.7	81.0	9.42	83.5	9.21
September ...	92.3	70.8	21.5	81.6	8.51	84.8	6.14
October ...	93.5	72.4	21.1	82.9	5.83	83.3	4.91
November ...	93.4	72.3	21.1	82.8	11.20	87.3	5.53
December ...	94.0	70.5	23.5	79.8	9.80	84.3	6.75

### *Trauma.*

Apart from those ulcers which are thought to be idiopathic, trauma plays an important rôle in the development of the lesion. Any trauma which breaks through the epidermis may initiate the development of tropical ulcer, but crushing blows, accompanied by haematoma formation, or lacerated cuts were the commonest type of injury. Even when antiseptic measures were taken soon after the injury, tropical ulcer often developed in such cases.

Two cases of dog-bite of the calf subsequently developed tropical ulcer on the site of the bites. Simple clean cuts, in some cases even if promptly treated, often break down and ulcerate. A variety of simple cut which may develop into tropical ulcer is that which is heavily painted with strong tincture of iodine and then bandaged up.

Less serious breaks in the continuity of the skin—scratches, abrasions

and punctures (caused by spines of plants, nails, etc.) are also starting points of tropical ulcer.

Cuts acquired on coral reefs by those vacationing in Tobago are frequently followed by ulcer-formation. It is alleged that cuts from living rather than dead coral are complicated in this way, but I can see no grounds for this belief.

Insect bites play an important rôle in the production of tropical ulcer. In this connection the commonest is from the insects known locally as sand-flies. Actually these insects are of the genus *Culicoides*—*C. amazonius*, *C. furens*, *C. stellifer*, and *C. diabolicus* (MYERS, 1935; ADAMSON, 1939). The vesicles arising from sandfly bites may be misinterpreted as being those of "idiopathic" tropical ulcer. Next in importance are mosquito bites (*Aedes* and *Anopheles*) and those of *bête rouge* (*Leptus batatus*). A more uncommon cause is penetration by the female chigoe (*Tunga penetrans*).

I have also seen tropical ulcer arise from lesions due to scabies (probably aided by scratching) and impetigo. It occasionally supervenes in lesions due to ringworm infections of the foot and in sores due to yaws.

### *Debility.*

Debility from other diseases has been held by many observers to be an important contributory factor in the etiology of tropical ulcer. Of these diseases, the most important are said to be malaria (CROSS, 1900; ASHLEY-EMILE, 1905; APOSTOLIDES, 1922), syphilis (KUTZ, 1912; SMITH, 1914; SOPRANO, 1914; KERSTEN, 1916; MENDELSON, 1921), scurvy (APOSTOLIDES, 1922), alcoholism and dysentery (ASHLEY-EMILE, 1905; SOPRANO, 1914) and ankylostomiasis (HUGHES, 1931).

Between 60 and 70 per cent. of the workers in Trinidad are infected with hookworm (in some districts the percentage is even higher), about 50 per cent. have malaria and a large but undetermined percentage have syphilis. Other debilitating factors are chronic gonorrhoea and avitaminosis. Diabetes mellitus is present in a small proportion of the East Indian cases. Among Europeans, chronic alcoholism is perhaps an influencing factor; I found tropical ulcer in Europeans commonest amongst white American oil-drillers, many of whom were addicted to strong liquor. It must be remembered, however, that the occupation of these drillers exposes them to blows, scratches from thorns and insect bites, rendering them more liable to infection than white clerks, laboratory technicians, etc.

### SYMPTOMATOLOGY.

#### *Acute tropical ulcer.*

The acute ulcer is usually oval or circular in outline; the margin is slightly raised above the surrounding skin and the edge slopes into the base at an acute

angle (as distinct from that of the syphilitic gumma, which meets the base at a right angle).

Covering the base is a slough, usually of a greenish-grey colour. This is fairly firmly attached to the underlying base and, if detached, produces extensive haemorrhages from the soft, pulpy granulomatous tissue which is thus revealed. The slough, in the newly-formed ulcer, fills it entirely, and may rise above the surface-level of the surrounding skin. As the ulcer ages the slough shrinks and in the chronic ulcer it may be entirely absent. The slough has a fetid odour which is quite characteristic.

In Trinidad I have never seen an ulcer extend into muscle, periosteum or bone, though this has been reported. I imagine that such complications would be prone to develop in races more primitive and isolated than the Trinidadians. In all my cases the process involved no more than the epithelium, subcutaneous tissue and fat.

The variety of acute ulcer known as the acute phagedenic ulcer which is fulminating in character and is associated with gross tissue destruction and frequently a fatal termination, was not encountered in my practice.

#### *Chronic tropical ulcer.*

This ulcer is a sequel to an untreated or unsuccessfully treated acute ulcer. The edge is heaped-up, pale and has a cartilaginous consistency; it forms a raised ring around the ulcer. The base is pink to almost white in colour and is devoid of a slough. Contrary to the findings of CLEMENTS (1936), I always detected a distinct and characteristic odour even in the chronic ulcer. Unlike that in the acute form, the base does not bleed readily and has a firm, hard consistency.

#### *The Papular stage (so-called Idiopathic type).*

The papular stage is held by some observers to represent an idiopathic form of tropical ulcer. The papules in question are, at the start, about 4 to 10 mm. in diameter, but they rapidly increase in size to a diameter of 1.5 cm. or more. When this diameter has been attained the dome of the papule breaks, revealing an ulcer which may further increase in diameter by an erosion of the margin.

In the majority of these cases I have been unable to exclude insect bites (especially those of *Culicoides*) as a cause of the papule. That the papule does, if untreated, develop into a tropical ulcer, is suggested by the following experience:—

Two children (one female, aged 6; one male, aged 7) were seen on the same day. They lived within 100 yards of one another and were playmates. Each was of similar racial origin and social status—the girl English-Venezuelan, the boy Scots-Mexican. Both had developed small papules on the shins, and the appearance of these lesions suggested a common infective origin. Cod-liver oil, applied locally, and M. & B. 693 by mouth, were prescribed. In

the case of the girl, these orders were carried out and the papules rapidly healed: the parents of the boy neglected my recommendations and a typical tropical ulcer developed on the site of one of the papules. The papules in these two patients closely resembled the "idiopathic" ulcers of some observers, it is possible that they were, in fact, ulcers of this type, but they might equally well have been produced by *Culicoides* bites.

### *Site and number of ulcers.*

I have never seen an ulcer above the level of the knee, but other observers have described lesions on the upper extremities and trunk.

The limitation of tropical ulcer to the lower extremity and to the particular sites listed in Table VI possibly depends on the fact that in these areas the terminal arterioles fail to overlap. As shown by RAUBER and KOPSCH (1912), the areas most often involved in tropical ulcer are those supplied by terminal vessels of adjoining arteries.

Perhaps another contributory cause of the high incidence of tropical ulcer in these areas is the fact that the adventitia of the arteries of the lower third of the leg has a rich supply of vaso-constrictor fibres (WOOLLARD and WADDELL, 1935); these fibres are more abundant in these areas than in the upper extremity, thigh or ankle-joint regions.

It is in the lower anterior tibial region also that a peculiar, hairless, depigmented and atrophied-looking skin occurs, in those suffering from vitamin B<sub>2</sub> deficiency (CLEMENTS, 1936; EARLE, 1941).

TABLE VI.

Site of Ulcer.	Percentage of total Cases.
Anterior surface of leg, lower half	69.2
Medial malleolus ... ..	14.3
Lateral malleolus ... ..	3.2
Lower part of tendo Achillis ...	6.2
Dorsum of foot ... ..	7.1

In this series of cases multiple ulcers occurred as follows: two ulcers in 9.5 per cent. and three ulcers in 9.5 per cent. Both lower extremities were involved in 6.2 per cent. of the above series.

### *Lymphatic enlargement.*

Although ABRAHAMS (1923) and BURNIE (1931) report that lymphatic glandular enlargement is frequent with tropical ulcer, I have not found this to be the case; a view also held by JIMENEZ (1928) and CLEMENTS (1936). In only one case did I see concomitant lymphadenitis—this occurred in the popliteal region and was followed by a popliteal abscess.

Recently, on Ocean Island, Central Pacific, I have seen about a score of so-called "tropical ulcers" in Chinese coolies. In each case there was lymphatic glandular enlargement (popliteal and inguino-crural), usually accompanied by abscess-formation. These ulcers, however, lacked the appearance of the West Indian type. They had a ragged margin, with undermining leading to other ulcers, had practically no slough and the characteristic odour of tropical ulcer was absent. I am of the opinion that the etiology of these ulcers is different from that of the Trinidad variety.

*Pain, pyrexia and constitutional disturbances.*

Pain is often very severe in the recent ulcer and this often leads to a neglect of the dressings (which are painful to change) and a high incidence of irregular attendance and default.

The chronic ulcer is usually almost entirely painless and quite rough treatment—irritant dressings, work, etc.—is readily tolerated.

I have seen neither pyrexia nor constitutional disturbances in my practice although both are said to be severe in the acute spreading type.

*Blood calcium.*

No estimations of blood calcium were made in my series, but findings obtained in Trinidad (PAWAN and CAMPS-CAMPINS, 1931) are given as a matter of interest; these are compared with those of other investigators in other parts of the world (BYRON, 1930; ORR and GILKS, 1931; BROWN, 1935). The table shown below was compiled by CLEMENTS (1936).

TABLE VII.

Observer.	Locality.	Class of Case.	Number of Cases.	Range of Blood Ca.	Average Blood Ca.
BYRON ...	Malaya Trinidad	Tropical ulcer	6	8.4-9.0	9.0
PAWAN and CAMPS-CAMPINS		"Anemia"	3	7.0-9.2	8.1
		Ulcer with debility	8	8.4-10.3	10.3
		Healthy ulcer cases	8	10.3-12.0	11.3
ORR and GILKS ...	E. Africa	Normal	24	9.5-12.2	10.7
		Prisoners and labourers	90	7.3-11.0	9.4
BROWN ...	W. Africa	Ulcer cases	57	7.0-11.2	9.7
		Europeans	13	10.7-12.5	11.2
		Phagedenic ulcers	54	5.5-15.0	9.52
		Non-phagedenic ulcers	45	6.3-15.0	9.58

Although evidence existed that the Trinidad labourer's diet is deficient in calcium (EARLE, 1941), in view of the conflicting findings listed above, there is no exact evidence as to the rôle, if any, of calcium deficiency in the production of tropical ulcer.

### *Microscopy of smears.*

In the acute type of ulcer, spirochaetes and fusiform bacilli were found in enormous numbers. The number of these organisms decreased with the age of the ulcer until, in the long-standing ulcer, it is difficult to find either type of organism. This is in agreement with the findings of CLEMENTS (1936).

### RELATIONSHIP OF TROPICAL ULCER TO ORAL SEPSIS.

CLEMENTS (1936) claims that infection of abrasions, cuts, etc., leading to tropical ulcer, results from saliva coming into contact with these skin lesions. The saliva may be deposited accidentally (promiscuous spitting), deliberately (being applied with leaves as a haemostatic) or on the feet or proboscides of non-biting insects (e.g., *Musca*). In the subjects investigated (natives of Manus, New Guinea), 25 per cent. suffer from either acute or chronic suppurative periodontitis (KIRKPATRICK, 1936). Smears from the gums of these people showed an abundance of fuso-spirochaetal organisms.

It is of interest to note that KING (1940) has recently suggested that Vincent's disease may be related to a deficient intake or utilization of nicotinic acid and its allied pyridine derivatives of the vitamin B<sub>3</sub> complex. Vitamin B<sub>3</sub> deficiency is common among Trinidadians (EARLE, 1941) and it might well be that because of this, growth of fuso-spirochaetal organisms takes place readily, either in the mouth or in skin lesions.

Unfortunately, I made no detailed study of mouth organisms in my practice, but I have already recorded the prevalence of pyorrhoea alveolaris, dental caries, etc., among Trinidad oilfield workers. (EARLE, 1941.)

Transmission of tropical ulcer by spitting cannot always be blamed for the spread of the infection. Expectoration is common enough in Trinidad, though less so than in betel-chewing communities, but many patients (Europeans, mixed creoles, Chinese) were in the habit of wearing shoes and socks. In such individuals the possibility of transmission by the finger-nails (from an infected mouth) or by biting or non-biting flies could not be excluded.

### SOME SPECIAL FEATURES IN FEMALE INFECTIONS.

In several cases I found that the ulcers, if under treatment, showed a marked exacerbation during the menstrual period. In untreated cases an increase in size of the ulcer may occur at this time. Several of the patients looked upon this as being perfectly natural, so the phenomenon is apparently well established in local folk-lore.

In one case, breakdown of the freshly-formed scar occurred during menstruation. Details are as follows :—

*Case history.*—R. C., negress, aged 35. First seen on 13th February, 1939, when she displayed a tropical ulcer of the left middle malleolar area of four weeks' duration. Apart from this lesion the patient was healthy. No abnormalities were found in the cardio-vascular, pulmonary, digestive or central nervous systems. The Wassermann reaction was negative ; no malarial parasites in the blood-film ; no hookworm ova in the stools. The urine contained no abnormal constituents.

Prontylin (*p*-aminobenzene-sulphonamide), 1·8 grammes daily, for 7 days, with cod-liver oil applied locally, was prescribed. On 20th February, the ulcer was smaller in size ; M. & B. 693 (2-sulphanilyl-aminopyridine) 3 grammes daily, for 7 days, was given. The improvement had been maintained on 27th February, and the ulcer was scarred over.

Seen on 3rd March, when the scar had broken down and the ulcer had re-formed. The menses had occurred since the previous visit and the patient volunteered the information that the ulcer had increased markedly in size during the last menstrual period, before she had consulted.

Certain observers have claimed success in the treatment of vaso-motor disturbances of the menopause by injections of ovarian follicular hormone. Accordingly, I gave injections of progynon B oleosum (benzoic acid ester of dihydro-follicular hormone), 10,000 international benzoate units every third day during the intermenstruum. This, continued with the other treatment (M. & B. 693 and cod-liver oil), tided the patient over the next menstrual period, and she made an uninterrupted recovery.

Unfortunately, lack of supplies of progynon prevented my making further tests on similar types of case.

## TREATMENT.

### 1. NATIVE REMEDIES.

(a) *Local applications.* Local applications are of two varieties—poultices and cold applications. As a poultice, the heated and split leaf of the *rachat* (a variety of prickly pear) is used. Banana leaf, either whole or cut up and made into a paste with water, is used as a cold application ; this, incidentally, favours the spread of the ulcer. Among Barbadians, vervein is applied to the ulcers. Extracts of the manchineel tree (*Hippomane mancinella*) have been applied to long-standing ulcers (BODEAU, 1936 ; EARLE, 1938).

(b) *Remedies by mouth.* Various bush-teas, infusions of herbs, etc., have been used in the treatment of the ulcers. They are, without exception, inert.

### 2. ORTHODOX TREATMENT.

(a) *Rest.* This is a very important factor in treatment. If absolute rest in bed can be secured the healing time is halved. Unfortunately, hospital



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### 2. ORTHODOX TREATMENT.

(a) *Rest.* This is a very important factor in treatment. If absolute rest in bed can be secured the healing time is halved. Unfortunately, hospital

beds are scarce in Trinidad, and patients ordered to rest in bed at home rarely do so as the crowded state of their houses leads to constant disturbance. Most of my patients, because of poverty, had to be treated as ambulatory cases: this greatly militates against any type of treatment.

(b) *Concomitant diseases.* The blood should be examined for malarial parasites and the Wassermann test should be performed. Stools should be searched for hookworm ova and the urine examined for sugar and the shreds of chronic gonorrhoea. Specific treatment for malaria, syphilis, hookworm, diabetes mellitus and gonorrhoea can be given at the same time as specific treatment for the tropical ulcer.

(c) *Dietetic.* Since avitaminosis  $B_2$  plays a rôle in the production of the disease, it is obvious that adequate amounts of this vitamin must be included in the patient's diet. The following foodstuffs, obtainable in Trinidad, are said to be rich in vitamin  $B_2$  (*Medical Research Council*, 1932; NICHOLLS, 1938): beef, liver, kidney, egg-yolk, milk, yeast, carrots, sweet potatoes, yams, bananas, dhol, English potatoes. In the case of Europeans, there is no difficulty in providing an adequate vitamin  $B_2$ -rich diet; but East Indians and negroes—either through negligence or poverty—could not be relied upon to carry out dietetic instructions. For such persons it was necessary to provide adequate amounts of yeast (marmite) daily.

In the case of alcoholics (Europeans) whose absorptive powers were defective (possibly owing to damage of the gastric mucosa by alcohol), it was necessary to give intramuscular injections of campolon (liver extract containing 10 R.U. of vitamin  $B_2$ —lactoflavin—and 10 R.U. of vitamin  $B_6$ —Györgyi's acrodynia factor or Goldberg's P.P. factor—per c.c.). This was conveniently administered in "depot dosages," i.e., one injection of 10 c.c. per week.

The prescribing of a diet rich in vitamin  $B_2$  did not, of course, preclude the taking of other food rich in the remaining vitamins or in minerals.

(d) *Local applications.* These are of two types: (1) Those designed to clean the ulcer and to remove slough, and (2) those intended to promote epithelialization of the ulcer.

Of the first variety I have used two: (a) magnesium sulphate and glycerine paste, and (b) copper sulphate solution, 1 in 150 (GUNTER, 1938). I soon discarded the first-named application as it was too painful and irritant. Copper sulphate solution does not give much pain and it cleans the base of the ulcer and removes the slough within 48 hours.

Following cleansing, applications promoting epithelialization must be used. Cod-liver oil as an application in this condition has already been described (COURTOIS, 1938; CORKILL, 1939; EARLE, 1940). If the cod-liver oil (cod-liver oil, 40 per cent.; vaseline, 60 per cent.) be applied under clastoplast and the patient is intelligent and can be kept reasonably isolated, this form of dressing is excellent. This is undoubtedly due to the high vitamin A content of the cod-liver oil, which promotes epithelial repair. Unfortunately, the very

objectionable odour of the ageing application, renders the patient a pariah to the sharers of his hut and bed, so that the treatment is often abandoned.

I found that whale oil was not nearly so unpleasant to use, and although its vitamin A content is not quite so high as that of cod-liver oil (*Medical Research Council*, 1932) the fact that it could be applied under elastoplast without fear of its being disturbed by the patient, made it much more convenient to use (DE DZIEMBOWSKI, 1934; EARLE, 1941).

Scarlet red ointment is of value in promoting final epithelialization in the few square millimeters of unhealed granulation tissue which sometimes remain stubbornly resistant to other types of application.

(e) *Spirochaeticidal drugs*. The presence of spirochaetes in tropical ulcer has naturally led to the employment of spirochaeticidal drugs by many observers. I have also tried these drugs in my early cases, but I soon abandoned them as useless, except in cases where syphilis co-existed. For the latter type of case drugs of both bismuth and arsenic groups were used. The bismuth group included bivitol (basic bismuth  $\alpha$ -carboxethyl  $\beta$ -methyl nonoate), rubyl (double iodide of bismuth and quinine) and casbis (activated bismuth hydrate). Among the arsenicals I used acetylarsan (diethylamino acetarsone) and myosalvarsan (sulpharsphenamine, sodium dioxy-diamino-arsenobenzene-methylene-sulphurous acid).

(f) *Sulphanilamide compounds*. The results of my experiences with this groups of drugs in the therapy of tropical ulcer have already been published. (EARLE, 1940.) Quoting from this paper "... the action of sulphanilamide compounds would appear to be doubtful in the case of long-standing ulcers, favourable in the case of recent ulcers and good in the pre-ulcerative or vesicular state. The value of the drug as a prophylactic in this condition remains to be proved."

Two types of sulphanilamide derivative have been tested, namely, M. & B. 693 (2-sulphanilyl-aminopyridine) and prontylin (p-aminobenzenesulphonamide). The daily dosage of M. & B. 693 was 3 grammes and of prontylin 1.8 grammes, for an adult. Under 12 years of age the daily dosage of M. & B. 693 was 2 grammes and of prontylin 1.2 grammes; under 6 years of age the daily dose was 1 gramme of M. & B. 693 and 0.6 gramme of prontylin. The tablets were given crushed, 4-hourly, and a sodium bicarbonate mixture was given concomitantly to minimize toxic effects.

### CONCLUSIONS.

1. Tropical ulcer occurs widely among the labouring classes in Trinidad and to a far less extent among the working and non-working European population.

2. It is rare in infancy, occurs after the 5th year, and attains its maximum incidence during the third decade—the decade in which the maximum amount of work is done.

3. Of the varied races in Trinidad, East Indians have the highest incidence of infection ; this is related to their inadequate diet and deficiency of vitamin B<sub>2</sub>.

4. The majority of ulcers occur during the period of low rainfall and low relative humidity. The reason for this is obscure.

5. The commonest traumata which result in tropical ulcer are crushing or bruising injuries and lacerated wounds. Insect bites play an important rôle, and these may well be the cause of the so-called " idiopathic " ulcer.

6. Predisposing diseases are hookworm, malaria, syphilis, gonorrhoea and diabetes mellitus. Alcoholism is a predisposing factor among Europeans and white Americans.

7. The acute ulcer resembles that seen in other countries, but it does not extend into muscle, periosteum or bone. The acute phagedenic ulcer is very rare in Trinidad.

8. The chronic ulcer is of the classical type and has a distinctive odour.

9. The idiopathic ulcer, commencing as a small papule, has, in my practice, never been proved to be dissociated from insect bites.

10. The majority of ulcers occur on the anterior surface of the leg, lower half. This may be due to poor arterial supply and the fact that this area is particularly affected by lack of vitamin B<sub>2</sub>.

11. Lymphatic glandular enlargement and constitutional disturbances are very rare with tropical ulcer in Trinidad.

12. Blood calcium findings are conflicting and give no definite conclusions.

13. The organisms obtained from the ulcer scrapings do not differ from those found in other countries.

14. The possibility of tropical ulcer being related to Vincent's infection of the mouth and of both these conditions being due to lack of nicotinic acid is indicated.

15. Tropical ulcer is unfavourably influenced by menstruation.

16. Native remedies are useless in this condition.

17. Rest, treatment of concomitant diseases and an adequate vitamin-rich diet are essentials of treatment.

18. Local application of cod-liver oil or, better, whale oil, is extremely valuable.

19. Arsenic and bismuth are useless in the treatment of tropical ulcer, but must be used if syphilis co-exists.

20. Sulphanilamide compounds are of value in the papular (pre-ulcerative) stage and in early ulcers, but they scarcely affect the long-standing ulcer.

#### SUMMARY.

1. A description is given of tropical ulcer, as it occurs in Trinidad.
2. Treatment of the condition is detailed.

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## STUDIES IN LEISHMANIASIS IN THE ANGLO-EGYPTIAN SUDAN.

### V.—CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS.

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#### INTRODUCTION.

During the past four years attention has been attracted to cases of dermal leishmaniasis occurring in various parts of the Anglo-Egyptian Sudan. A number of the lesions recently observed have presented certain interesting features not hitherto recognised; these have been described in a series of previous communications (KIRK, 1938; KIRK and DREW, 1938; KIRK and SATI, 1940a, 1940b) in which analogies have been suggested between the Sudan conditions and various forms of dermal leishmaniasis seen in India and elsewhere. A general review of the facts relating to dermal leishmaniasis in the Sudan indicates that the subject is by no means as straightforward in this country as it appears to be in some of the other endemic centres of leishmaniasis where anomalies of geographical distribution have rendered it easier to differentiate the various types of infection.

The present communication is an attempt to summarize the facts which have been so far elucidated, and includes also some observations on the curious form of oral leishmaniasis occasionally encountered in the Sudan. For purposes of analogy or contrast reference is made to conditions of leishmaniasis occurring

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in other parts of the world, but the study is concerned primarily with leishmania infections observed in the Sudan. Hence it does not follow that any of the suggestions tentatively put forward are applicable in the other endemic centres of leishmaniasis.

The study is restricted in its scope to infections contracted in nature by human beings, and is therefore essentially clinical. It may be added that attempts to throw further light on the subject by studies in laboratory animals have proved disappointing. Hamsters have not so far been available for experimental work, and the results of inoculating the parasites into other animals, including monkeys, have been so variable as to be useless for purposes of the present inquiry.

#### TYPES OF DERMAL LEISHMANIASIS.

Clinically the most important consideration in any condition of dermal leishmaniasis is to determine whether the infection is purely cutaneous or whether the skin lesions are part of a generalized infection, involving also the abdominal viscera and other tissues. With few exceptions it is generally easy to classify the dermal lesions observed in the Sudan into one or other of those two main categories. For descriptive purposes the two main categories may each be further subdivided into two, thus giving four principal types of dermal leishmaniasis which may be grouped as follows:—

##### A. PURELY CUTANEOUS INFECTIONS.

1. Oriental sore (ulcerating).
2. Non-ulcerating "leishman nodules."

##### B. CUTANEOUS MANIFESTATIONS OCCURRING IN THE COURSE OF A GENERALISED INFECTION.

3. Skin lesions in untreated kala-azar (generally ulcerating).
4. Cutaneous manifestations apparently produced as a result of successful treatment of visceral kala-azar (non-ulcerating).

In spite of an apparent etiological basis the suggested classification is primarily clinical. The present writer has not encountered any instance of the infections referred to in Group 2. He has, however, seen numerous examples of all the other types, and it is his opinion, that with the possible exception of those in Group 2, the four principal types of dermal leishmaniasis which have been enumerated are clinically distinct, and can be easily differentiated.

It will be observed that this provisional classification does not include the dusky pigmentation of the skin which is so frequently seen on the face, hands, feet, and abdomen of kala-azar patients. The cause of this pigmentation is somewhat uncertain at present, but the condition is not considered to be strictly comparable with the cutaneous affections described in the present paper.

## ORIENTAL SORE.

It has been shown (KIRK and SATI, 1940a) that ulcerative cutaneous manifestations are not infrequently found in cases of Sudan kala-azar. This makes it very difficult to decide whether a condition of cutaneous leishmaniasis occurring in the kala-azar areas is due to oriental sore or is a cutaneous manifestation of kala-azar. In Baghdad, where kala-azar does not occur, it seems safe to assume that all dermal leishmaniasis are due to oriental sore. In North China oriental sore appears to be absent, but skin lesions occur in dogs affected with kala-azar, and possibly also in human beings, since CASH and HU (1927) have found that the skin is frequently involved in kala-azar cases, even in the absence of definite lesions. In India, diagnosis is made easier by the fact that the two diseases appear to have a different distribution; but in other places, such as Transcaspia and the Sudan, confusion easily arises on this point. To overcome this difficulty we have provisionally adopted an arbitrary method of differentiation. A condition of dermal leishmaniasis occurring in a proven case of kala-azar is regarded as a cutaneous manifestation of the latter condition. In the absence of proven kala-azar it is similarly regarded if the patient has fever, or enlargement of either liver or spleen. The difficulties of diagnosis under these circumstances are illustrated by the following histories:—

## CASES.

1. *Mohd. Ahmed, male, aet. 16.*

Complained of fever for one month with epistaxis. An ulcerating sore, about the size of a two-shilling piece present on the anterior surface of the middle third of the left leg, said to have appeared 14 days before the onset of the fever. Spleen palpable eight fingers' breadth, liver four fingers' breadth. Blood negative for malaria. No leishmania seen in peripheral blood, nasal smears, sternal and splenic puncture, but they were found in a scraping from the leg ulcer, and in a puncture from the related femoral glands. The case was regarded as kala-azar and treated accordingly. The fever settled somewhat with two courses of neostibosan, but a low irregular pyrexia still persisted, so blood was taken for agglutination tests and was found positive (1:2,500) against the organism of Malta fever. Thereafter the patient was put on to sulphonamide. The spleen which had already decreased in size, was no longer palpable and the fever subsided. At this stage he was discharged, although the liver was still palpable. Nine months later the patient was seen in his village: the spleen had re-enlarged to the level of the umbilicus, and he was obviously a very ill man, but refused to return to hospital.

2. *Fatma Ali, female, aet. 28.*

Complained of fever, headache, and blood-stained diarrhoea of a month's duration. Liver palpable four fingers' breadth and tender, spleen four fingers' breadth. Ulcerating sore present on the forehead, said to have appeared before the onset of the fever; it had the typical "bouton" appearance of oriental sore, and a scraping from it revealed leishmania. Spleen and sternal puncture were both negative for leishmania. Blood: R.B.C., 3,340,000, W.B.C., 4,200; differential count: P. 34 per cent., L. 52 per cent., L.M. 14 per cent., Eos. 0. The stool contained *E. histolytica* (cysts) but a course of emetin had no effect on the fever. Although the blood was negative for malaria a course of quinine was given, but without effect. Thereafter splenic and sternal puncture were repeated, with negative results, and a third splenic puncture carried out after a provocative dose of neostibosan (cf. HENDERSON, 1937) was also negative. Agglutinations against typhoid, paratyphoid and Malta fever were negative. The patient was regarded as a clinical case

of kala-azar,\* and treated accordingly. After one course of neostibosan the fever subsided, and the spleen began to diminish, but the patient insisted on leaving hospital at this stage, and has not been seen since. At the time of leaving hospital there was no evidence of healing in the sore on the forehead.

A diagnosis of oriental sore *sensu strictu* seems to be justified only in a case where the cutaneous lesion occurs in a patient who remains afebrile, and in whom no evidence of visceral or oral infection can be made out clinically. Infections which were regarded as oriental sore because they satisfied those criteria have already been described from Darfur and the Blue Nile area (KIRK and DREW, 1938). Even in such cases the possibility always exists that the cutaneous lesion may represent a primary sore which might at a later date have been followed by a generalized infection but for the specific treatment undertaken since, when monkeys are inoculated subcutaneously with leishmania from cases of Sudan kala-azar, the development of the visceral disease is sometimes preceded by the appearance of a primary skin lesion of the oriental sore type at the site of inoculation (ARCHIBALD, 1922). In this connection the following case which has been observed recently is of some interest:

#### CASE

The patient was a European lady who had travelled widely during the past few years in all parts of the Sudan, including the kala-azar areas. In May, 1940, two small sores appeared on the dorsum of the left hand; they were comparatively inconspicuous, and not much attention was paid to them, but they showed no tendency to heal. In September, 1940, the condition was seen by the present writer, and typical leishmania were found in smears from the sores. During this period there had been no history of fever, and there was no evidence of visceral involvement. Specific treatment was withheld as it was hoped that carbon dioxide snow would shortly be available, by means of which treatment could be made entirely ambulatory. This, however, did not become available, and in December, 1940, the sores healed spontaneously, no specific treatment having been given in the meantime. There has been no subsequent indication of visceral involvement, although 18 months have now elapsed since the appearance of the sores.

#### DISTRIBUTION OF ORIENTAL SORE.

We have previously described (KIRK and DREW, 1938) typical cutaneous infections of the oriental sore type from Darfur and the Blue Nile areas, and similar infections observed in the past (cf. KIRK, 1939) have possibly been contracted in Shendi and Khartoum, both of which lie outside the kala-azar zone. During the past two years a number of cases have been found by Dr. IBRAHIM ABBAS in Kadugli, a town in the Nuba Mountains district. The *Annual Report of the Sudan Medical Service for 1938* records a case of cutaneous leishmaniasis in Equatoria, but it is not stated whether there was any evidence of visceral involvement also in this instance or not.

#### LEISHMAN NODULES.

Many years ago THOMSON and BALFOUR (1910) described "two cases of non-ulcerating oriental sore, better termed leishman nodules," which they

\* At the time this patient was seen gland puncture had not yet been introduced as a diagnostic method.

had observed in Khartoum. In neither case was there any clinical evidence or history of a visceral infection. The lesions were multiple, subcutaneous, slowly developing nodules simulating keloids, with no tendency to ulceration. A heavy infection of leishmania was found in material from the nodules. The patients were two Egyptian soldiers who had recently come from that country to Khartoum, and it is probable that the infection was contracted not in the Sudan but in Egypt where several relatives of one of the patients were said to have had the same disease. About the same time FERGUSON and RICHARDS (1910) observed a number of similar cases in Egypt, and also verrucous and granulomatous dermal leishmania infections which they described under the same name of "parasitic granuloma." Shortly afterwards ARCHIBALD (1911) described a similar condition in a Nuba soldier, in whom there was likewise no evidence or history of visceral involvement, and it is almost certain that this infection originated in the Sudan. These old records are not without historical interest, and they attracted a certain amount of attention at the time; on account of the somewhat anomalous features of the lesions, BRUMPT (1913) regarded the causal parasite as a new species, which he named *Leishmania nilotica*.

Eleven years later BRACHMACHARI (1922) published the first description of "post kala-azar dermal leishmaniasis" from India in which he showed that the dermal condition was a sequel to visceral kala-azar which had been successfully treated with antimony. During the next few years other similar cases were observed in India. In a paper describing a series of such cases, ACTON and NAPIER (1927) pointed out the close similarity between the leishman nodules of THOMSON and BALFOUR and some of the lesions in their own Indian cases. They suggested that the former were in all probability examples of post kala-azar dermal leishmaniasis, although the detailed notes published by THOMSON and BALFOUR give no indication of visceral infection, and no history of treatment. It may be noted, however, that ACTON and NAPIER had observed instances of dermal leishmaniasis in which there was no history of previous treatment for kala-azar, and consider the dermal condition in these cases to be the sequel of a visceral infection which ended in spontaneous recovery without being recognised. In several such cases they elicited the past history of an obscure febrile illness, sometimes associated with splenic enlargement, which had subsided with the spontaneous decline of the fever.

In the course of observations extending over the past five years, the present writer has not observed "leishman nodules" in the Sudan, except in cases of treated visceral kala-azar. A lesion somewhat resembling those described by THOMSON and BALFOUR, but occurring on the leg, has been described in two cases as a post kala-azar condition following treatment with antimony (KIRK and DREW 1938, KIRK and SATI 1940b) and we have recorded elsewhere (KIRK and SATI, 1940c) the development of typical "leishman nodules" in another case after treatment with 4:4' diamidino stilbene. In the latter instance

the lesion, which appeared in the supraclavicular region was almost an exact replica of those described by THOMSON and BALFOUR.

It may be, as ACTON and NAPIER suggest, that the cases described by THOMSON and BALFOUR were instances of post kala-azar dermal leishmaniasis, following spontaneous recovery from an unrecognised visceral infection. This view, however, is largely speculative. The infections were probably contracted in Egypt, and if the same hypothesis be applied to the other similar cases reported from Egypt it would suggest a prevalence of kala-azar in that country which is not substantiated by the published records, and require some modification of present views on the distribution and lethality of African kala-azar. In the absence of any further indication to the contrary, there seems no reason at present to regard the condition as other than a purely cutaneous infection. Non-ulcerating types of oriental sore have been described by CARTER (1911) in India, and also by observers in other parts of the world. In this connection an old observation relating to oriental sore in Bagdad may be of some interest. STURROCK, who practised in that city for 4 years informed MANSON (1917) that in rare instances oriental sore may recur more than once, but as a rule the sores of the second attack do not ulcerate.

#### CUTANEOUS INFECTION IN KALA-AZAR.

In a previous paper (KIRK and SATI, 1940) it was shown that cutaneous infections occur in a noticeable proportion of cases of Sudan kala-azar, but in the clinical description of the lesions no differentiation was made between those which are found in cases of untreated kala-azar and those which are apparently produced as a result of treatment. The remarks comprised in this section of the present paper apply exclusively to lesions of the former type.

The lesions are generally ulcerating in character, but they may be very inconspicuous. Clinically they appear either as a superficial, scabbing, lupus-like condition (KIRK, 1938) or as circumscribed ulcers varying from the size of a split lentil to that of a florin piece ( $\frac{1}{2}$  to 4 cm.), usually superficial in character, frequently multiple, and sometimes with a tendency to coalesce. Sometimes the ulcer is slightly raised above the surrounding skin, and covered with hard adherent scabs, just like the classical illustrations of oriental sore. They occur most frequently on the exposed parts of the body, and apart from the demonstration of visceral involvement, differ in no way clinically from the lesions described in a previous section of this paper as purely cutaneous infections.

It cannot be determined whether these lesions represent primary reactions at the sites of inoculation or are merely part of the generalised infection. Some cases give a history of the skin condition being noticed before the onset of the fever, but little reliance can be attached to this, and it is well known that, in experimental animals (HINDLE, 1928) and in canine kala-azar, infection of the skin may be a prominent feature of the generalized infection, sometimes in

the absence of noticeable lesions. Infection of entirely normal areas of skin has not, so far, been demonstrated in Sudan kala-azar patients, although as we have stated the lesions are often exceedingly inconspicuous and might easily pass unnoticed.

#### DERMAL ERUPTIONS APPEARING AS A RESULT OF TREATMENT.

In a previous communication in this series (KIRK and SATI, 1940b) a description was given of a punctate cutaneous eruption commonly found in cases of kala-azar in the Sudan, and appearing during treatment. It was shown that the rash occurs in cases treated with aromatic diamidines as well as in cases treated with antimony, and hence cannot be regarded as a specific toxic effect of antimony, but is more probably a manifestation of the leishmanial infection, although the mechanism concerned in its production is still obscure. In addition, attention has been directed (KIRK and DREW, 1938; KIRK and MACDONALD, 1940; KIRK and SATI, 1940c) to certain more conspicuous skin eruptions which have been observed to appear during treatment in a number of cases of Sudan kala-azar; leishmania can usually be found in eruptions of the latter type, and the illustrations which have already been published show the close similarity between the lesions and the various forms of "post kala-azar dermal leishmaniasis" described by ACTON and NAPIER (1927) and other workers in India, although their relation in time to the recovery from kala-azar is slightly different.

It is the opinion of the present writer that the different types of skin eruption which have been described as occurring in cases of Sudan kala-azar during treatment are all manifestations of the same phenomenon, differing from each other in degree only, but not in their essential nature. They all appear under the same circumstances, and go through essentially the same course, while a complete series of gradations from the most inconspicuous punctate rash to florid nodular or verrucous eruptions can be observed in different cases, and sometimes actually in a single case. The main features of these eruptions are given below, and the remarks embrace all types of cutaneous lesions which appear in Sudanese kala-azar patients during treatment.

*Time of onset.* The eruptions usually appear towards the completion of treatment, or in the first week or two after the patient's discharge from hospital as cured. This is in contradistinction to the post kala-azar skin conditions observed in India which usually appear 1-2 years after cure of the visceral disease. The writer's experience in the Sudan suggests that the development of such eruptions during treatment has a good prognostic significance as far as the cure of the visceral disease is concerned, indicating not necessarily that cure has been completed, but that the case will ultimately progress to a satisfactory recovery. The following case is of interest in that the diagnosis of kala-azar was finally confirmed by the appearance of a nodular eruption after treatment.

## CASE

*Ahmed Adam, male, aet. 35.*

Admitted with fever, spleen and liver both enlarged. *Blood.* R.B.C. 4,212,000, W.B.C. 3,600 ; differential count : P. 42, L. 49, L.Mn. 9, Eos. 0. Splenic puncture was done on five separate occasions but leishmania were not found. The formol-gel test was negative. Sternal puncture, nasal smear, throat swab, and peripheral blood were all negative for leishmania. Widal reactions against typhoid, paratyphoid and Malta fever were negative. Although the blood was negative for malaria or other parasites, a course of quinine was given, but without effect. The case was diagnosed as "clinical kala-azar"\* and given a course of neostibosan. This caused some decline of the fever ; the temperature did not, however, settle completely, so a course of solustibosan was given, at the end of which the fever settled finally, and a generalized nodular eruption appeared, most prominent on the face. Leishmania were readily found in scrapings from this rash. With a view to treating the rash, a course of tartar emetic was given, without effect, and later a course of urea stibamine. This had little effect on the rash, but the liver was now non-palpable, and the spleen only just palpable, so the patient was discharged from hospital. When seen 9 months later, he was very well. Spleen and liver were both not palpable and the rash had almost disappeared, only a few punctae on the forehead remaining.

## CLINICAL APPEARANCE.

The eruption is always most prominent on the face, where it appears first, usually on the forehead or malar region. Sometimes its distribution is restricted to those regions, but most commonly the whole face and neck become involved, sometimes also the trunk and even the limbs. The following clinical types occur : (1) Minutely punctate rash ; (2) papular eruption ; (3) nodular eruption ; (4) verrucous or papillomatous eruption.

Sometimes a minutely punctate rash is all that appears. Papular, nodular or verrucous eruptions generally occur on the face, and are frequently preceded by a minutely punctate rash in the same situation, which becomes exaggerated into the more florid types. When this happens, the more florid eruptions on the face are usually accompanied by a punctate rash on the neck and trunk, the individual lesions becoming less conspicuous as they are followed downwards over the trunk. Occasionally discrete nodular or papillomatous lesions occur on the legs. Ulceration does not occur ; there are no subjective symptoms, such as itching, and in the less conspicuous forms, the eruption may pass unnoticed by the patient until his attention is directed to it by the medical attendant. Nodular or papillomatous eruptions on the face, on the other hand, generally attract attention, and when these appear after discharge from hospital the patient generally comes back, rather concerned to find out what has happened to him.

*Course of the eruption.* In contradistinction to the post kala-azar dermal leishmaniasis of India, it can be said that, in a number of cases at least, the skin eruptions produced by treatment in Sudanese kala-azar patients may disappear spontaneously after about 6 months. This appears to be the case whether the eruptions were produced by treatment with antimony or by aromatic

\* Gland puncture had not yet been introduced as a diagnostic method.

diamidines. In nodular or papillomatous forms the disappearance of the lesions may be associated with a scaly desquamation.

In a number of cases the cutaneous manifestations produced by treatment may pass through a stage of depigmentation, before they finally disappear, and this is of considerable interest. Occasionally minute depigmented areas can be observed accompanying the punctate rash which we have described as the earliest manifestation of this cutaneous condition, and analogy with the macular depigmented rash (NAPIER and DAS GUPTA, 1930) of Indian post kala-azar dermal leishmaniasis is exceedingly suggestive in such cases. In Sudan cases, however, depigmentation is essentially a secondary change, which is preceded by nodular or punctate eruptions, and occurs as those subside. Moreover the depigmented areas may be very much larger than the punctate lesions which preceded them, and very much more conspicuous. The depigmented patches shown in Fig. I were preceded by a minute punctate rash, so inconspicuous that it was recognised only by two observers. A little girl who developed similar depigmented patches after treatment in Sennar was nicknamed "Nimr" (the leopard) by the other patients because she was so conspicuously spotted.

Leishmania have not so far been demonstrated in the depigmented areas. The return to normal pigmentation in these areas seems to be influenced by exposure, since it occurs more rapidly in the face and arms than in the trunk.

The following interesting case illustrates an extreme example of this:—

#### CASE.

*Derwanis Melas, male, aet. 40, a merchant in Singa.*

Twenty-one years ago the patient developed kala-azar in the Singa district and was treated by Dr. V. S. HODSON, in Khartoum hospital, where he remained five months and received a large number of injections. At the end of treatment he states that he developed a rash all over his body, especially prominent on the face. After his discharge from hospital as cured, the rash became scaly and the scales fell off, with the aid of soap and water, leaving depigmented areas, which have persisted over a period of 20 years, except on his face and forearms. During this period there has been no indication of relapse of the visceral disease. At the present time the patient is well nourished and healthy. Neither liver nor spleen are palpable. Pigmentation is normal in the exposed parts of the body, *i.e.*, the face, neck and forearms, but all over the trunk are conspicuous depigmented areas, most of them slightly larger than a shilling, very similar to those shown in the case in Fig. 1.

#### OBSERVATIONS ON ORO-NASAL LEISHMANIASIS.

From time to time cases of oro-nasal leishmaniasis are observed in the Sudan, and some of the cases, especially those which come under observation in an advanced condition (cf. SUSU, 1914) closely resemble the advanced stages of the disease known as espundia (leishmaniasis americana). There are, however, certain notable differences. In the first place, true espundia has a very specific course of evolution in the human subject, which has not so far been traced in any Sudan case, *viz.*, the appearance on the exposed parts of the body of one or more primary lesions of the oriental sore type, which heal



leaving characteristic scars, and are followed after a period of months or years by the appearance of intractable eroding ulcers of the buccal or nasal cavities. In the second place, oro-nasal leishmaniasis is relatively uncommon in the Sudan as compared with kala-azar, and occurs only sporadically; it has never been observed in epidemic form, affecting a large proportion of the population as in certain districts of South America. Moreover, it may be noted that the Sudan is not the only old world centre of leishmaniasis in which sporadic oro-nasal infections have been observed. A similar disease has been found in those parts of Italy where oriental sore is endemic (MANSON-BAHR, 1921). A form described as Indian oro-pharyngeal leishmaniasis was recorded many years ago by CASTELLANI and CHALMERS (1919), while more recently NAPIER and DAS GUPTA (1930, 1934), DASTIDAR (1939) and other workers have depicted ulcerative conditions of the lips, palate and tongue as clinical varieties of post kala-azar dermal leishmaniasis.

During 8 years service in the Sudan, the present writer has seen fourteen cases of oro-nasal leishmaniasis. These have all been patients under the care of colleagues in the Sudan Medical Service, often in different parts of the country. Only in two instances has it been possible to follow up the cases during treatment and subsequently. In most cases the writer has seen the patient on one or two occasions only, but through the kindness of his colleagues he has had access to the clinical records, and been able to examine all cases personally.

The lesions vary somewhat in different patients. Generally, however, they conform to one or other of the following principal types:—

(a) Lesions of the gums, palate, and fauces, varying in character from swollen purplish patches to diffuse, exuberant, and often ulcerating granulomata. (Fig. 2.)

(b) Ulcerative granulomata inside the nasal cavities, causing swelling or bulbous appearance of the nose.

(c) An ulcerative lesion, involving the nasal septum, alae nasi, and surrounding skin.

(d) A condition resembling cheilosis with some localised swelling and ulceration, usually of the lower lip. (Fig. 3.)

In all cases typical leishmania were found. Frequently they were scanty or absent in direct smears from the ulcerating surface, and only found by excising a piece of more healthy (that is to say, non-ulcerating) tissue from the margin of the ulcer and making smears from this. Occasionally they have been observed in paraffin sections of the granuloma.

There were no primary cutaneous sores, or histories of such. In two cases there was definite evidence of visceral infection, and in many of the others there was at least suggestive evidence of this disease. The writer's clinical records of the fourteen cases are naturally scanty, but the data below are of some interest.



FIG. 1.—Depigmented stage of post kala-azar skin eruption.



FIG. 2.—Oral leishmaniasis. Diffuse granuloma of palate.

MAYNE (1935). Not only therefore does the oro-nasal leishmaniasis of the Sudan differ clinically and epidemiologically from leishmaniasis americana but doubts may be expressed whether it should be regarded as a separate disease occurring apart from kala-azar.

A case has been described (KIRK and MACDONALD, 1940) in which an intra-nasal ulcer containing leishmania appeared simultaneously with a nodular cutaneous eruption after treatment with antimony, thus suggesting analogy with the post kala-azar oro-nasal lesions reported from India by NAPIER and DAS GUPTA (1934). All cases, however, do not follow this course, and it would appear from the observations we have recorded that oro-nasal leishmaniasis in the Sudan is not uncommonly associated with evidence of concurrent visceral involvement. It looks as if the parasites of kala-azar have a special tendency in some instances to attack also the mucous membranes of the oro-nasal cavities, just as certain strains of oriental sore produce a generalized infection in white mice which is followed by secondary cutaneous lesions around the ears, tail and scrotum (PARROT, 1928).

This view receives some support from a number of studies which are undertaken with an entirely different object in view. FORKNER and ZIA (1934) showed that leishmania could often be found in nasal and tonsillar smears from kala-azar cases in China. Their observations have been confirmed in the Sudan by ARCHIBALD and MANSOUR (1937), HENDERSON (1938), and in a small unpublished series of cases by Dr. MANSOUR and the present writer. In the latter series, diagnosis was actually made in two instances from nasal swabs before splenic puncture was undertaken, but there is little to recommend nasal swabbing as a routine diagnostic procedure. Positives are found only in a small proportion of cases, while the microscopic search for leishmania among the varied flora and debris of a nasal smear is a tedious and doubtful undertaking, beset with many puzzles and fallacies. Occasionally, however, leishmania may be found in large numbers, and in one such case we were able to show that the parasites were viable and infective, since a monkey (*Cercopithecus aethiops*) was infected by breathing the spray from a de Vibliss atomizer containing a saline emulsion of the patients' nasal secretion. Tonsillar swabs from 50 cases of kala-azar in Singa revealed leishmania in one instance only; the infection, however, was a heavy one, although the tonsils appeared to be perfectly normal.

#### COMMENTS.

It has for many years been accepted that the diseases known as kala-azar, oriental sore, and espundia are produced by separate varieties of *Leishmania* which have been given specific names. Furthermore, the parasite of Indian kala-azar is generally regarded as a species distinct from the parasite of Mediterranean kala-azar. The causative organisms of kala-azar in China, the

Sudan, Transcaspia, and South America occupy an indeterminate position in this classification.

In the Sudan the position is of peculiar interest. Kala-azar, oral leishmaniasis and dermal lesions resembling oriental sore may all be found in the same endemic area. The course of the Sudan visceral disease in the human subject is not identical with that of either the Mediterranean or the Indian varieties. Neither *Phlebotomus perniciosus* nor *P. argentipes* have been found in the Sudan, where there is evidence that a different vector is concerned, so it may be assumed that the parasites responsible for kala-azar in the Sudan are not identical with either the Indian or the Mediterranean varieties.

Modern pathology tends to differentiate strains of parasitic pathogens rather than species, and recent work on malaria indicates that this conception can be usefully applied in the case of protozoal infections as well as to bacteria and filterable viruses. As with the malaria parasite, there are possibly many different strains of leishmania, adapted to local varieties of the vector, and varying in virulence for the human beings and animals; possibly, in the case of the leishmania, varying also in their selective affinity for different tissues, some tending to dermatropism, others tending to viscerotropism, and yet others having a special affinity for the mucous membranes of the oro-nasal region. Possibly also the reaction of the host plays a part in determining the final result of an infection, since even in oriental sore cases are occasionally observed in which obscure febrile attacks have aroused the suspicion of transient generalized infection (MANSON, 1917).

It would appear that there are strains of leishmania in the Sudan comparable to oriental sore, in that they seem to cause purely cutaneous infections, without visceral involvement. At present it is uncertain whether oro-nasal infections in the Sudan are associated with a special type of parasite or not. Unless we have encountered a remarkable series of double infections, the observations in the present paper suggest that the parasites of Sudan kala-azar may at times produce also cutaneous and oro-nasal infections.

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## RELAPSING FEVER IN ABYSSINIA.

BY

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All the cases in this series occurred at Soddu in the Lake District of Abyssinia during the period from July 2nd to August 19th, with the exception of one patient, a Mukamba, who was seen at Giggiga on 1st September. Soddu lies at an altitude of 7,500 ft. and has a cold climate with a heavy rainfall. As the local Abyssinian natives are often heavily infested with lice, conditions are ripe for serious epidemics of relapsing fever. The patients comprised African soldiers from the Gold Coast, local Abyssinian civilians and one Mukamba. It is regretted that in some cases the notes are fragmentary, but this was partly due to the fact that out-patients did not always return at specified times for re-examination.

### CLINICAL SIGNS.

Only those patients who showed spirochaetes in their blood slides are included in these observations. There were thirty-two such cases.

The onset, although acute, was not so abrupt as in the case of such a disease as lobar pneumonia. There was usually a history of symptoms for 1 to 4 days before admission to hospital. The average history for the military patients was 2 to 5 days. The soldiers, whose symptoms were usually confirmed by a medical officer's report, gave more reliable histories than the Abyssinians. Two of the most prominent signs were (1) an enlarged and tender liver and (2) dyspnoea.

The hepatic symptoms were present in nearly every case. The liver was enlarged and tender in twenty-three cases, including all the Gold Coast patients. There was marked tenderness below the right costal margin. Actual palpation of the liver was usually impossible owing to the extreme tenderness, often combined with rigidity, but the extent of the enlargement could be detected by percussion. There was usually only an enlargement of one or two fingers' breadths below the costal margin, but in one case the liver nearly reached the level of the umbilicus. There was no increased-percussion dullness upwards into the chest. These symptoms, although improving as soon as the other signs had begun to subside, usually persisted to a lessening extent for from 10 days

\*I am indebted to Brigadier R. E. BARNSELY, M.C., Director of Medical Services, G.H.Q., East Africa Command, for permitting me to publish this article.

to 2 weeks. Jaundice was present in five cases and was severe in three of them. All the jaundiced patients had heavy spirochaetal infections.

Dyspnoea was a symptom in ten out of thirty-two cases. This was sometimes accompanied by a slight dry irritating cough. There were no signs in the chest except a few rhonchi in two patients. A striking feature was the severe respiratory embarrassment, which resembled that of lobar pneumonia and was diagnosed as such before admission on one occasion, combined with the complete absence of physical signs in the thorax. The respiratory rate reached 62 in one patient and 46 in another case. One patient showed slight blood staining of his sputum for 24 hours. In another case red blood cells were seen on microscopic examination of his sputum.

Enlargement of the spleen was not such a prominent sign nor was it so often or so markedly tender as the liver, but it was usually palpable (in nineteen cases) and often tender (in thirteen cases). One patient suffered severe pain in the region of his spleen which persisted for 2 months and was so intense that it woke him up at night. In his case there was not only tenderness below his left costal margin but also on pressure of his eighth to eleventh ribs from mid-axillary to posterior axillary lines.

Diarrhoea was present in two cases.

Conjunctivitis was evident in two cases. Cerebral symptoms occurred in one patient. He suffered from delirium, subsultus tendinum, incoherent talking and spasmodic movements of his arms. His pupils were contracted and reacted to light. He died 2 days after admission.

Neuritis was a complication in four cases. It occurred during convalescence. The symptoms were neuralgic pain and local hyperaesthesia in the affected part. The time of onset, distribution and duration are shown in Table I.

TABLE I.  
NEURITIS IN CASES OF RELAPSING FEVER.

Time of onset after admission.	Distribution.	Duration.
Case 1, 4 days ...	Subcutaneous surface of right tibia.	5 days.
Case 1, 9 days ...	Outer surface of right fibula.	15 days.
Case 2, 3 days ...	Lower part of front of right thigh.	1 week.
Case 3, 10 days ...	Subcutaneous surface of tibiae. Bilateral with simultaneous onset in both legs.	Still painful, though improving, after 16 days when he was discharged.
Case 4, 10 days ...	Subcutaneous surface of tibiae. Bilateral with simultaneous onset in both legs.	Still painful, though improving, after 9 days when he was discharged.

Deafness, which was present on admission, occurred in one patient, but no abnormal signs were found on auroscopic examination. His hearing gradually improved and was nearly normal when he was discharged after being in hospital for  $2\frac{1}{2}$  weeks.

### BLOOD EXAMINATION.

As is usual in relapsing fever elsewhere, there was commonly an increase in the polymorphonuclear leucocytes. Differential counts were performed before treatment on twenty-three cases, including six children, two of whom were aged 6 months, two aged 2 years, one aged 12 years and one 13 years. Thirteen cases, including two children aged 13 years and 2 years respectively, showed a raised polymorphonuclear count of over 70 per cent. Seven patients had counts of over 80 per cent., one being as high as 89 per cent. Eight cases, including three children aged 6 months in two cases and 2 years in one case, had polymorphonuclear counts below 60 per cent. As a rule a heavy spirochaetal infection was associated with a high polymorphonuclear count, and a mild infection with a low or normal count. There were, however, six exceptions to this rule. Neither the number of spirochaetes nor the character of the differential count appeared to be associated with any change in the severity of the disease or ultimate prognosis. There was usually a rise of monocytes after treatment, which varied from 12 to 20 per cent. in six out of seven cases examined (all adults), and a reduction in the number of polymorphonuclear leucocytes within 2 or 3 days of the injection. It is unfortunate that, owing to the absence of a haematocytometer, it was impossible to perform total leucocyte counts and thus obtain exact figures for the various types of white cell.

### ARSENIC TREATMENT.

#### EFFECT OF ARSENIC INJECTIONS ON THE NUMBER OF SPIROCHAETES IN THE BLOOD.

In view of the fact that better clinical results appeared to arise from initial fairly large intravenous injections than from smaller doses by the same route, it was decided to ascertain to what extent the dose and route of injection affected the rate of disappearance of the parasites.

(1) *After an injection of \*Neoiacol 0.6 gramme intravenously.*—Of six cases examined at approximately 4-hourly intervals after the injection, five showed no spirochaetes in thick films after 8 hours, and the other showed a few spirochaetes after 8 hours but none after 11 hours. Another patient still showed a positive slide after 6 hours but had no spirochaetes when next examined 11 hours

\* Neoiacol is an Italian arsenical preparation. It is issued in two forms :—

- (1) For intravenous use with the formula "diossidiaminoarsenobenzolo monometan—solfinato di sodio."
- (2) For intramuscular use with the formula "4,4'—diossi—3,3' diamino—arsenobenzolo solubilizzato e stabilizzato."



after the injection. It is therefore probable that the majority of cases develop negative slides about 7 hours after the injection. One exception occurred in the case of a patient whose blood was not negative until 19 hours after the injection.

(2) *After an injection of Neoiacol 0.3 gramme intravenously.*—It was only possible to examine one patient; his blood slide showed a fairly large number of spirochaetes after 10½ hours, but no parasites when next examined 20 hours after the injection.

(3) *After Neoiacol intramuscularly* according to the approximate dosage of 0.01 gramme per each kilogram of body weight. Three patients were examined. In all cases spirochaetes were still present in the blood after 24 hours. Two of the patients were examined after 36 hours, one showing a positive and the other a negative slide. All were negative after 48 hours.

These figures, although too small to be conclusive, suggest that fairly large doses of arsenic intravenously act more quickly in ridding the blood of spirochaetes than either similar doses by the intramuscular route or smaller doses intravenously.

The arsenic acts by causing a progressive diminution in the number of spirochaetes as shown in Table II. In order to obtain some kind of comparative estimate of the numbers of spirochaetes in the blood, fifty leucocytes were counted together with all the spirochaetes which occurred in the same field. The injection in each of these cases was neoiacol 0.6 gramme intravenously.

TABLE II.

Case number.	Spirochaetes per 50 Leucocytes.			
	Before injection.	4 hours after injection of neoiacol.	8 hours after injection of neoiacol.	11 hours after injection of neoiacol.
1	138	36	26	0
2	218	92	0	
3	24	15	0	

#### EFFECT OF ARSENIC ON THE MOTILITY OF SPIROCHAETES.

It was found that spirochaetes remained actively motile for several hours (usually 17 to 24 hours in this series) in a fresh coverslip preparation of blood, as there was little tendency for the serum between the corpuscles to dry up in the cold climate of Soddu. An attempt was therefore made to ascertain whether arsenic acted on the spirochaete by diminishing its activity, or by rendering it more delicate so that it would die more quickly than spirochaetes which had not been exposed to the drug. The following are figures giving the duration of motility in blood taken before and at different periods after the injection of neoiacol 0.6 gramme intravenously:—

(1) *Blood taken before injection.*—Eight cases were examined. Spirochaetes were motile for 8 hours in one case, 17 hours in two cases, 18 hours in three cases, 22½ hours in one case and 24 hours in one case.

(2) *Blood taken 5 minutes after injection.*—Three cases were examined. Spirochaetes were motile after removal of blood for 8 hours in two cases and for 18 hours in one case.

(3) *Blood taken 6 hours after injection.*—Two cases were examined. Spirochaetes were motile after removal of blood for 16 hours in one case and for 12 hours in the other case.

(4) *Blood taken 17 hours after injection.*—One case was examined. The spirochaetes were motile for only 2 hours.

These results do not give much information, for even after exposure to the action of arsenic in the body for as long as 6 hours the spirochaete may still remain active for many hours. There was, however, a tendency for the organisms to remain motile for a slightly longer period in blood taken before than after injection, but the figures are too small to be of great value. It is interesting to note that the spirochaetes in a fresh coverslip preparation, taken 6 hours after an intravenous injection of 0·6 gramme neoiacol, usually retained their motility for some hours after the time when a thick film from the same patient had been found free from spirochaetes. This is shown by a comparison of the above figures with Table II.

#### *Morphology of the Spirochaete.*

The morphology could be well studied in the fresh specimen because the organisms became gradually more sluggish and could therefore be more easily observed. Its shape was quite different from that seen when stained with Leishman's or Giemsa's stain. Instead of being twisted into various shapes, as in stained slides, the spirochaete had an axis which was straight and spirals which were absolutely regular. Most of the organisms were very long and contained from five to fifteen spirals. These characteristics still persisted even after the parasite had ceased to be motile.

#### EFFECT OF ARSENIC ON CLINICAL CONDITION.

(1) *Dose of 0·6 gramme intravenously* or the appropriate dose for a child. The temperature nearly always falls permanently to normal within 1 to 4 days (in 16 out of 18 cases), but this gives a false impression of the rapidity of improvement. There was a definite, often great, improvement within 24 hours and a very marked benefit in 48 hours. The pulse dropped to 90 or below that level within less than 48 hours after the injection in 12 out of 17 cases. There was not a single relapse among the eighteen non-fatal cases, the average period of observation after injection being 22 days.

(2) *Arsenic in smaller doses.*—The following cases gave a typical clinical picture of relapsing fever, but owing to the absence of microscopic equipment in the Field Ambulance at that time, the diagnosis was not confirmed microscopically before treatment. The blood of all these patients, when examined

during the later stages of their pyrexia after specific treatment had been administered, showed no spirochaetes. The patients had been treated with doses of 0.3 or 0.45 gramme novarsenobillon intravenously which were sometimes repeated if there was no beneficial effect. The standard of a satisfactory response is taken to be a permanently normal temperature.

(a) 0.3 gramme *Novarsenobillon intravenously*.—Two cases were seen. In one case the temperature reached normal after 7 days, a repetition of the injection having been given on the 3rd and 6th days. There were then 2 days of normal temperature, followed by a return of pyrexia with diarrhoea and dilated heart for 4 days. The patient was very jaundiced and developed haematuria and bleeding gums. He improved and was transferred, but I heard later that he had died 3½ weeks after the onset of the disease. The second case "responded" after 8 days.

(b) 0.45 gramme *Novarsenobillon intravenously*.—Five cases were examined. Two "responded" in 5 days. Two cases "responded" in 10 days, but one of these patients had received a second injection of 0.6 gramme on the 5th day, and the other case suffered a 10 days' relapse of fever after 4 days of normal temperature. The fifth case developed a normal temperature only after 11 days, in spite of a repetition of the dose on the 4th day.

The prolonged course of these cases treated with smaller doses contrasts with the comparatively rapid benefit produced by the larger injection of 0.6 gramme organic arsenic. A repetition of smaller doses does not seem to produce the same satisfactory response as one big injection. It appears that smaller doses, while ridding the peripheral blood of spirochaetes, fail to arrest the symptoms of the disease.

#### CAUSE OF CLINICAL SIGNS.

It is possible that the dyspnoea may result from the aggregation of spirochaetes in the lung capillaries, just as cerebral signs are said to be due to a similar condition of the cerebral capillaries. Hepatic signs and diarrhoea may also be caused by the collection of large numbers of the parasites in the affected organs. Spirochaetes, apparently identical morphologically with *Sp. recurrentis*, were found in the sputum of two patients, but in view of the fact that spirochaetes are liable to be found in the sputum under other conditions, I hesitate to give an opinion as to whether the organisms were of the relapsing fever variety. One of the cases revealed, on microscopic examination of his sputum, a few spirochaetes, some red cells and pus cells 20 hours after an intravenous injection of neoiacol 0.3 gramme. The thick blood film was at this time negative for parasites, but his blood had shown a heavy infection before the arsenical injection. The other case clinically resembled relapsing fever, with dyspnoea as a prominent symptom, although his blood slide was negative for spirochaetes. It may be that the absence of positive slides in association with a typical clinical course of

relapsing fever, of which I saw one other case, or with the persistence of symptoms after a small arsenical injection, is explained by the confinement of spirochaetes to certain internal organs.

#### OTHER CLINICAL TYPES.

*Abortive Cases.*—Four cases, as shown in Table III, occurred during this epidemic of Gold Coast soldiers with tender enlarged livers, frontal headaches, furred tongues, repeatedly negative blood slides for malaria and relapsing fever and with only mild pyrexia.

TABLE III.

Case No.	History.	Maximum rise.			Duration of pyrexia.	Differential leucocyte count.
		Temp. °F.	Pulse.	Resp.		
1	3 days	101	92	28	7 days	Polymorphs. 48; lymphocytes, 30; monocytes, 16; eosinophils, 6.
2	3 days	99	96	22	1 days	
3	4 days	101	92	22	3 days	
4	2 days	100	99	22	3 days	Polymorphs. 29; lymphocytes, 37; monocytes, 7; basophils, 1.

Three cases also occurred of catarrhal jaundice with marked jaundice, bile in urine, tender palpable livers, negative blood slides and mild pyrexia, with a maximum rise of temperature to 99.6°, and pulse to 88, which persisted for a period varying from 2 days to 12 days. These cases may have had no connection with relapsing fever, but the occurrence of so many cases of hepatitis and catarrhal jaundice in a small military population of about 1,000 (for all these patients were Gold Coast soldiers) during a period of less than 7 weeks may be of significance when taken in conjunction with the definite hepatic signs of the relapsing fever subjects. The small polymorphonuclear count in Case 4 (Table III) is difficult to explain on the theory of a relapsing fever origin, though the differential count of Case 1 resembles those of microscopically confirmed relapsing fever patients a few days after arsenical treatment. It is possible that the spirochaete with its predilection for attacking the liver so lowered the resistance of this organ that it predisposed to the outbreak of an independent epidemic of infective hepatitis in the community.

#### Course.

A study of the disease amongst the Abyssinian out-patients who had received no previous treatment and of those soldiers who had been given small

arsenic injections suggested that the pyrexial course is longer than that usually shown by other types of relapsing fever. The usual duration of one continuous bout of pyrexia in an untreated case is probably about 10 days.

### *Mortality.*

Out of thirty-two cases with positive blood slides there was one death. This series included fifteen Gold Coast Africans, sixteen Abyssinians and one Mukamba.

### DIAGNOSIS.

It was found that the most rapid method of diagnosis was by examination of fresh coverslip preparations of blood. As spirochaetes were in most cases numerous, it was usually possible to confirm the diagnosis within about 2 minutes. This method was of great value in Abyssinian out-patients work and obviated the possibility, which had often previously occurred, of patients failing to return for the result of stained slides. When fresh blood examination was negative a thick film was taken. By using small thick films and careful spacing one can examine the blood of over a dozen patients on the same slide, with great economy in stain and slides, an important factor in Field Ambulance work.

### *Intermediate Host.*

The louse, *Pediculus corporis*, was proved to be the vector by the discovery of spirochaetes in two of these insects. In one case the infected louse was caught on the patient's clothes and in the other case the louse was on the patient's out-patient card. The spirochaetes were found in the contents of the ruptured gut, which also contained disintegrating human blood cells. It is interesting to observe that whereas both lice were heavily infected, the patients' blood slides in both cases contained such small numbers of spirochaetes that they were only found with difficulty, in spite of the fact that the blood slides were taken at the same time as the removal of the lice. It seems probable, therefore, that multiplication of the spirochaetes had taken place in the insects' guts.

### SUMMARY.

1. In a series of cases of relapsing fever in Abyssinia hepatitis and dyspnoea were the outstanding clinical signs.
2. Differential leucocyte counts revealed an increase of monocytes and a diminution of polymorphonuclear leucocytes within a few days of the arsenic injection. The differential count appeared to have no prognostic significance.
3. Intravenous injections in fairly large doses acted more rapidly in removing spirochaetes from the peripheral blood than either intramuscular or smaller intravenous injections.
4. There is a progressive diminution in the number of spirochaetes in the peripheral blood after injection, as revealed by 4-hourly blood slides.
5. Motility tests were carried out. The spirochaetes in specimens of blood taken 6 hours after injection were still motile at a time when thick films revealed no parasites.

6. The shape of the spirochaete in a fresh blood preparation differed from that in a stained slide.

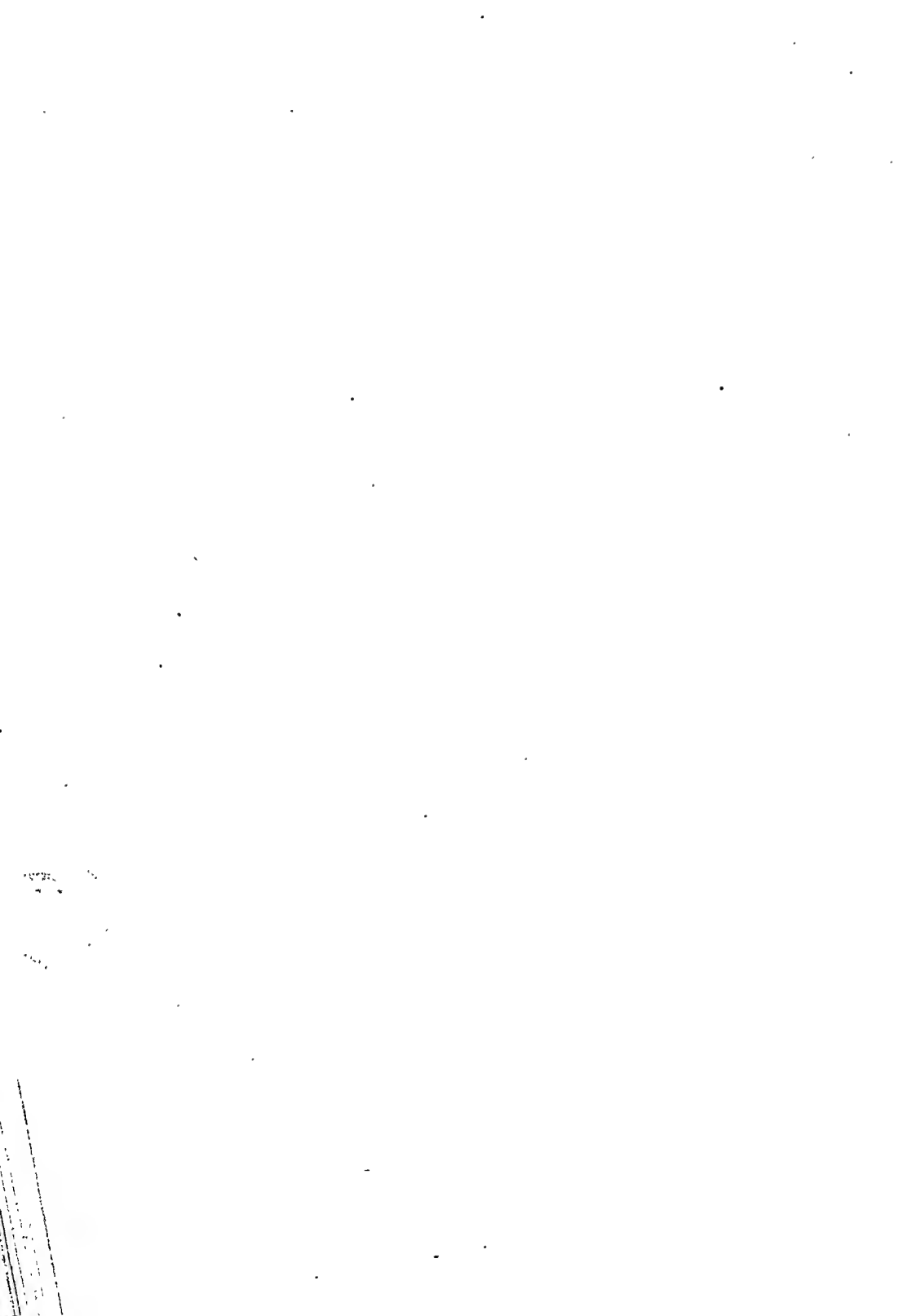
7. The intermediate host was proved to be the louse, *Pediculus corporis*. Larger numbers of spirochaetes were found in the gut contents of the lice than in the blood films of the patients.

8. Other cases of hepatitis occurred during the time of this epidemic. The possibility of their connection with relapsing fever is discussed.

9. The pyrexial course tended to be of longer duration than that of other types of relapsing fever.

10. A satisfactory diagnostic technique for the large out-patient clinic consisted of examination of fresh blood and simultaneous staining of multiple thick films on the same slide.

11. It is important to administer a large initial dose of arsenic intravenously; small injections had little beneficial effect even when repeated, and were sometimes followed by relapses. No relapses occurred after the larger doses.



## THE DIAGNOSIS OF BILHARZIASIS IN SOUTHERN RHODESIA.

BY

MICHAEL GELFAND, M.B. (CAPE TOWN), M.R.C.P., D.M.R.\*

*From the Public Health Laboratory, Salisbury, Southern Rhodesia.*

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The diagnosis of bilharziasis as a rule offers no great difficulty, especially in the vesical form, when the patient himself often notices blood in his urine. In fact, before the disease was recognised in Rhodesia, the haematuria was known by the lay people as "red-water."

In addition to the haematuria, which is often terminal, pain and frequency of micturition, hypogastric pain or discomfort, or not infrequently pain in the lumbo-sacral region, radiating down the thighs, may be noted. These symptoms would naturally cause the clinician to send the patient's urine to the laboratory to be examined for ova, the presence of which would be sufficient proof that the disease is present. In practically all cases the ova are terminal-spined (*Schistosoma haematobium*).

The urine may be a clear red or more often, when less blood is passed, smoky. Specimens with ova always contain albumin, and red blood corpuscles are found on microscopical examination. This has been noticed in Egypt and is borne out by my experience. If any particular specimen does not contain albumin ova will not be found in it. The amount of albumin varies—often

\* My thanks are due to Dr. RICHARD MORRIS, Acting Medical Director, for permission to publish this paper.



there is only a trace, but at other times there is a large quantity. It is therefore unnecessary to examine a specimen for ova if albumin is not present. Sometimes when albumin and blood are found in a specimen no ova are present. It is essential that the examination be repeated at least six times, as often ova will be found in one of the later specimens.

In cases of haematuria or albuminuria, where bilharziasis is strongly suspected, and no ova are seen, a cystoscopy is advisable. The presence of bilharzial inflammation will be easily recognised by the characteristic sandy-grain appearance of the bladder mucosa.

#### *Case Example.*

A young soldier, prior to entering the army, had periodic attacks of haematuria, and had lost weight. At no time were ova found in his urine, although albumin and blood were present: there were no casts. The blood pressure was normal and there was no oedema about the eyes. A cystoscopy was performed, when the necessary evidence of bilharziasis was found. He was given a course of antimony and since then there has been no recurrence of these symptoms.

The differential diagnosis of vesical bilharziasis is concerned with other diseases causing albuminuria, haematuria and frequency of micturition, such as nephritis, calculus and tumours in the urinary tract or kidneys, orthostatic albuminuria, pyelitis and cystitis.

The intestinal form of bilharziasis, usually due to *S. mansoni*, may be very difficult to diagnose. Even though the bowel is involved by an inflammatory process the patient very rarely has attacks which can be described as true dysentery. In most cases bowel symptoms are slight. Blood, evident to the naked eye, may be entirely absent, or it may be passed in such small amounts as to escape the notice of the patient.

So far I have met only one case which could have been mistaken for a case of dysentery.

The history is as follows: The patient was a lieutenant in the Rhodesian Forces. His condition was diagnosed as amoebic dysentery, although at no time were amoebae found in his stools. He had had bouts of dysentery for 10 years. At the beginning of the war he was sent to North Africa, where he had another attack in which the motions consisted entirely of blood and mucus. He returned to Salisbury. When I saw him this attack had passed off. I had the stools carefully examined and these were found to contain large numbers of ova of *S. mansoni*. He was given a full course of intravenous antimony. He began to put on weight and his general feeling of lassitude completely disappeared.

Except for periodic attacks of diarrhoea, generally mild, or some abdominal discomfort and vague pains, there may be nothing in the history to suggest intestinal bilharziasis. Very often the stools of patients who are not well and losing weight are sent to the laboratory for routine examination. In such cases ova are frequently found.

I think sigmoidoscopy is of value in suspected cases. Lesions produced by the ova must be present in the rectum or colon. The only drawback is that the inflamed areas are not as characteristic as the sandy-grain appearance

of the bladder. Most cases known to be suffering from intestinal bilharziasis investigated so far by sigmoidoscopy in the Salisbury Native Hospital, have revealed the presence of small haemorrhagic areas in the mucosa. Ulceration and papillomatous formation appear to be uncommon, especially the latter. In suspected cases, therefore, the finding of these haemorrhagic or congested foci in the bowel may be sufficient evidence to justify treating the patient with antimony, provided amoebiasis is carefully excluded.

In occasional cases the main complaint may even be constipation.

#### *Case Example.*

A young man of 24 had suffered from constipation for a few years. He had lost weight especially in the last 9 months prior to my examination. He felt tired and complained of flatulence. At no time had he noticed blood in his stools. Specimens of urine and stool were examined but no ova were found. The blood, however, revealed an eosinophilia of 13 per cent. This finding, together with his history of exposure to rivers since childhood, led us strongly to suspect bilharziasis. I had the specimens repeated, and eventually ova of *S. mansoni* were found in the stool.

The differentiation from amoebiasis may cause some difficulty, but the finding of the vegetative forms or cysts of the entamoeba will help to differentiate in most cases. Finally, the sigmoidoscopic appearances are quite different. Typhoid is readily excluded by its acute onset and pyrexia and by blood culture or serological investigations. Similarly, bacillary dysentery is characterized by its sudden onset and severe constitutional symptoms, together with the finding of the causative organism on culture of the stool. Malaria may on occasions disturb the bowel function and cause diarrhoea. The presence of parasites in the blood, however, readily distinguishes it from other conditions.

There are certain features in bilharzial cases which may lead one to suspect the presence of this disease when urinary or intestinal symptoms are completely lacking. In the absence of other diseases these signs and symptoms should lead the clinician to suspect bilharziasis.

The first essential in Southern Rhodesia is to determine whether the patient has exposed himself to infection. A large number of rivers in the Colony are infested with snails—either *Physopsis africanus* or *Planorbis pfeifferi*. A patient who has never come into contact with any of these waters cannot have bilharziasis. It is necessary to point out here that exposure to a stream does not necessarily mean only bathing in it. Washing the hands in it or drinking from it, is just as dangerous. Most people can recall whether they have been so exposed. Even one exposure may result in the contraction of the disease.

A most important feature of bilharziasis is an early loss of weight. This may be obvious to the patient's friends or may be noticeable only if he weighs regularly.

#### *Case Example.*

This occurred in a young woman of 27 years. She began to lose weight, and this caused her friends to remark about her health. She was working in the laboratory with infected snails. A blood examination revealed an eosinophilia. This continued to rise. No ova were found in the urine or stool, probably because the disease had not yet localised

itself in the bladder or rectum. She was given a course of antimony and the result was truly remarkable.

At least half the bilharzial cases I have met with so far have complained of dyspepsia. There may be abdominal pain after food and this is often epigastric. Heartburn and flatulence may also be experienced. These may in fact be the patient's only symptoms. From the history it will be noticed that the pains are irregular and do not show the constancy of those found in peptic ulcers. In one case where flatulence was the main complaint I did a cholecystogram, expecting to find evidence of cholecystitis. I later found *S. mansoni* in the stool.

#### Case Example.

A native boy in the laboratory saw me about epigastric pains occurring after meals. These had troubled him for 2 or 3 weeks. He had lost much weight. I examined his urine and found ova of *S. haematobium*. He received the necessary treatment and is now very well.

Apart from loss of weight and epigastric pain, another prominent symptom may be a feeling of lassitude, tiredness or debility. The patient may find concentration difficult. Not infrequently such cases are confused with neurosis.

#### Case Example.

The patient was a sergeant in the Army, 25 years of age. He had been appearing at sick parade for about six months. The only illness in his previous history was malaria. He was given anti-malarial treatment, although his blood smears were always negative. I was asked to see him to determine whether he should be recommended for discharge from the Army: it was uncertain whether he was malingering or neurotic. He told me that he had lost much weight. His appetite was poor and he had no energy. He found physical training a strain. He was born in Rhodesia and had swum in the rivers for a number of years. Examination revealed him to be rather thin. The spleen was markedly enlarged. There was no eosinophilia but the blood sedimentation rate (B.S.R.) was slightly raised—7.5 per cent. I examined his blood for evidence of malaria but without success. His urine was clear but ova of *S. mansoni* were present in the stool. He was admitted to hospital and after a course of antimony his general health improved considerably.

This case also illustrates two points—the negative history of intestinal symptoms and the splenomegaly that may be associated with infection by *S. mansoni*, of which mention will be made later.

Much faith is placed in the estimation of the eosinophilia. It is true that bilharziasis often causes an eosinophilia, especially in its early stages, but later this is often absent. In my experience only a third of the cases, when first seen by the doctor, show an eosinophilia of varying intensity. A normal differential count does not exclude bilharziasis. Eosinophilia, on the other hand, provided all other causes—especially those due to helminthic infections such as ankylostomiasis or allergic states—are excluded, is suggestive of bilharziasis. An eosinophilia, particularly when there is a history of exposure, should cause one to examine the urine or stool most carefully for the presence of ova.

Recently I have attempted to estimate the blood sedimentation rate in this disease. If bilharziasis, when untreated, were invariably to result in a rapid sedimentation rate, then a normal drop in a suspected patient would of necessity

exclude this possibility. I selected twenty-three cases known to be suffering from this disease; thirteen of these showed an increase in the sedimentation rate, the remainder being normal. Of these thirteen cases, eight showed a marked increase, two cases reaching levels over 50 per cent., and the remaining five were slight (6-10 per cent.). Therefore 43 per cent. were normal and 57 per cent. showed an increase. Just as an eosinophilia should make one persist in the examination of the excreta for ova, so I feel should a rapid B.S.R.—provided other conditions are excluded—make one careful not to overlook bilharziasis.

The following case illustrates the absence of an eosinophilia in the blood but an increased B.S.R.

The patient was a boy aged 12 years. For the past year he had noticed haematuria. He had swum in many rivers. The eosinophil count was 3 per cent., but the B.S.R. was 10.3 per cent. Investigation of his urine showed ova of *S. haematobium*.

On the other hand there may be a normal sedimentation rate, but a definite eosinophilia as revealed in the following case:—

A male, aged 23, felt off colour for a few years and had lost much weight. There was nothing in his history to point to involvement of bowel or urinary tract. The B.S.R. was 1 per cent., but the eosinophil count was 17 per cent. The latter finding made us suspect bilharziasis as he had frequently exposed himself in the rivers. After repeated investigations of the excreta, *S. mansoni* was found in his stool.

Bilharziasis in a few cases may produce a pyrexia which may last several days or even continue for a few weeks. Such cases may in fact be confused with typhoid or undulant fever. I think it is always of value after all tests for the usual causes of pyrexia—such as typhoid, undulant fever, tuberculosis or a suppurative process in the body—are negative, to consider the possibility of bilharziasis.

#### *Case Example.*

The patient was a young native male aged about 14 years. He was admitted to hospital because of loss of weight, debility and generalised pains in the body. His temperature was of a swinging character of moderate degree. The spleen was considerably enlarged. Blood smears were negative for malaria parasites. Tests for enteric and abortus fever were negative. He was given quinine but with no effect. The urine and stool contained bilharzia ova. After about 10 days I decided to give him antimony, and within a few days the temperature had settled. His general health improved considerably towards the end of the treatment.

I have outlined above some of the general manifestations of bilharziasis. Also, in this colony, involvement of certain organs or viscera should cause one to consider the possibility of the disease. The bladder and large bowel have already been mentioned. Other important organs which may be affected are the liver, spleen, appendix, Fallopian tube, testicle and the lungs.

The presence of an enlarged liver, especially if the surface is nodular and the edge hard, is strongly suggestive of bilharziasis. It has long been established in Egypt, Japan, Nyasaland and other African territories, that bilharziasis, especially in the intestinal form, may lead to cirrhosis of the liver and splenomegaly. The extent of enlargement of the liver or spleen may vary enormously.

The spleen may be grossly enlarged whilst the liver, though cirrhotic, may not even be palpable. At postmortem I have often found cirrhotic livers and demonstrated histologically the presence of bilharzia ova and tubercles in them, although up till now I have never found ova in the spleen.

The clinical manifestations of this condition differ in no important respect from the ordinary case of Laennec's cirrhosis of the liver due to such causes as alcohol. In many cases the main features are ascites and oedema of the legs. In others jaundice may be marked. In not a few instances this hepatolienal syndrome may be found although the patient presents himself with a totally different complaint such as a fractured limb. Death may eventually ensue from a terminal pneumonia, a severe haemorrhage, cholaemia or not infrequently in primary malignant disease of the liver. Other causes of an enlarged liver such as are due to alcohol or syphilis should naturally be excluded. Malaria does not produce a cirrhotic liver, although in the past this syndrome or a splenomegaly was often attributed to malaria.

#### *Case Example.*

A European male, aged about 35 years, was admitted to hospital complaining of a cough. He had lost much weight and his appetite was poor. He had been a non-drinker for many years. He had frequently bathed in Rhodesian rivers. On examination an enlarged, hard liver was felt, the edge being about 3 inches below the right costal margin. The spleen was much enlarged and its lower border reached to about the level of the umbilicus. The Wassermann reaction was negative. His urine was clear but ova of *S. mansoni* were found in the stool.

Bilharziasis may produce a chronic inflammatory change in the appendix. In about a quarter of the cases an acute super-imposed inflammation occurs, probably the result of stricture formation in the already inflamed appendix. The symptomatology varies greatly while in a large number of cases there are no symptoms at all. At postmortem chronic bilharzial inflammation of the appendix is often found, although death was due to a totally different cause. If symptoms are present they take the form of pain in the right iliac fossa. Tenderness will usually be found in this region. There is unfortunately no certain means of establishing the diagnosis pre-operatively. It may be suspected if the patient is known to be suffering from bilharziasis. Often an appendix is removed where bilharziasis is thought to be the cause, but no pathological lesion suggestive of this disease can be demonstrated. The difficulty in diagnosing chronic bilharzial appendicitis is due to the fact that various lesions may occur in other structures related to the right lower abdomen such as the ureter, caecum, mesentery and glands, or in women, the ovary and Fallopian tube, all producing much the same symptomatology. On account of this difficulty, one should be reluctant to diagnose chronic bilharzial appendicitis. On the other hand, when the appendix is acutely inflamed the symptomatology is characteristic and early removal is necessary even without deciding whether bilharziasis is present or not.

Bilharzial salpingitis can only be suspected after excluding other causes of salpingitis such as gonococcal or those following upon childbirth. In an uncomplicated case there is no leucorrhoea and the tender swollen appendages can be felt in Douglas' pouch or in one or other fornix. A previous history of exposure to rivers and the finding of ova in the urine or faeces will no doubt strengthen the suspicion of bilharzial salpingitis.

Bilharzial orchitis is not uncommon, especially in the native. The diagnosis is arrived at mainly by excluding carefully other causes of testicular swellings and the finding of ova in the urine. Not only may the body of the testicle be involved but also the epididymis. The surface of the swelling may be irregular due to bilharzial tubercle formation. Syphilis, tuberculosis or gonorrhoea offer no difficulty, but it may be impossible to distinguish it from a seminoma of the testis. In the latter case only histological examination after orchidectomy will reveal its true nature.

Bilharzial involvement of the lungs is seen in about 25 per cent. of natives suffering from the disease. There may be no symptoms, but usually there is a chronic cough. Asthmatic attacks due to bilharziasis have been reported in the past, especially in Egypt. Bronchial asthma is certainly not uncommon in the native, but whether bilharziasis may account for some of these cases I am unable to say definitely.

In a few cases the resultant fibrosis may be so advanced as to lead to shortness of breath and subsequent right ventricular failure, producing a condition not unlike that of Argerza's disease.

#### *Case Example.*

This patient was a native aged about 40 years. He was admitted to hospital with signs of congestive cardiac failure and died within a few days. At autopsy the right ventricle was considerably dilated and both lungs were extensively fibrosed throughout. The liver was congested. The bladder mucosa showed the typical sandy-grain appearance. Histological examination of the lungs showed that the fibrosis was due to bilharziasis. Numerous healed bilharzial tubercles were present in the fibrosed tissue. On digestion of a portion of the lung in caustic potash, ova of *S. haematobium* were recovered.

While the clinical examination in a patient suffering from chronic cough often reveals little of note, yet radiological investigation of the chest will show definite changes in 25 per cent. of cases. These include prominent hilar shadows with fibrotic striations radiating from them, especially towards the middle and lower zones of the lungs. Small irregular foci may be seen at times. There should be no need to confuse these cases with pulmonary tuberculosis as the radiological features are quite different and bacteriological examination of the sputum is negative.

It will be a great step forward when complement-fixation or skin tests are introduced in Rhodesia. It would be of value in the diagnosis of the many doubtful cases we meet and also a means of discovering how effective the treatment has been.

## SUMMARY.

Bilharziasis is a chronic inflammatory disease. The symptoms and signs may therefore be general or constitutional such as fever, loss of weight, lassitude, an increased blood sedimentation rate, or a blood eosinophilia.

Involvement of certain organs such as the liver and spleen, appendix, Fallopian tubes, testis and especially the bladder and bowel should always make one consider the possibility of bilharziasis in this territory.

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## CORRESPONDENCE.

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### CONGENITAL IMMUNITY IN TYPHOID FEVER.

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*To the Editor, 'TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

It might seem as though my paper on typhoid fever\* claims that there is a congenital immunity to typhoid fever. My paper does not prove this, but suggests it as a cause of an apparent relative freedom from the disease up to 4 years of life and as a cause of an apparent lesser toxicity from the disease should it occur at such an early age. There may of course be some explanation other than a congenital immunity for the facts that I mentioned but so far no other cause has come to my notice. The importance lies in the decision as to whether children of such early age should be inoculated against typhoid; inoculation reactions are usually severe, and, as huge numbers of children in endemic countries are malnourished, severe reactions might well terminate in disasters such as pyaemia or cardiac beriberi.

The idea of congenital immunity may be wishful thinking, but I think it is useful unless disproved.

I am, Sir, etc.,

J. V. LANDOR.

*General Hospital,  
Singapore.*

*9th October, 1941.*

\* LANDOR, J. V. (1941). Typhoid fever: with special reference to the value of new antisera in therapy and eosinopenia in diagnosis. *Trans. R. Soc. trop. Med. Hyg.*, 35, 1.



## DIAGNOSIS OF KALA-AZAR.

*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In a recent communication DAVIES & WINGFIELD\* have discussed the various methods of diagnosing kala-azar; smears from splenic, sternal hepatic and gland punctures. Each of these methods has its advocates, but it should be emphasized that there are a relatively small but nevertheless important number of cases of Mediterranean visceral leishmaniasis which would be missed in a single examination by any of the above methods because of the relatively few parasites in the viscera. In spite of the small number of parasites these cases may be clinically severe and show an intense anaemia. These cases can be readily diagnosed by culturing juices obtained by puncture. We have also frequently found that in experimental laboratory animals, infections overlooked by examination of spleen smears were detected by culturing splenic juice.

Since a patient must be subjected to the real inconvenience of splenic or other puncture *he should invariably be given the benefit of having the juice of any organ punctured sown on several tubes of suitable medium.* If this rule were adopted as a routine few if any cases could escape diagnosis and the necessity for repeated puncture would be avoided.

I am, etc.,

S. ADLER.

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Jerusalem.*

\* DAVIES, A. & WINGFIELD, A. (1941). Agranulocytosis in kala-azar and use of adrenalin. *Trans. R. Soc. trop. Med. Hyg.*, 35, 421.

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COMMUNICATIONS.\*

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A NEW TREATMENT FOR DRACONTIASIS.†

BY

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*Capt., Royal Army Medical Corps.*

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HISTORICAL.

The eradication of guinea-worms from the tissues has for many years been the subject of conjecture as witness the following quotation taken from the biography of that remarkable French explorer RENÉ CAILLIE (1799-1838). He writes : "The native name for this agonising complaint is translatable as 'the misery.' It is said that this humble admirer of the human race pushes her way into our bodies with drinking water. She is no fatter than a horse hair. Once she gets in she wants to get out again, and makes for the open air through the skin, often the skin of legs or feet. This produces a sore so tender that sufferers howl with the pain of it. Should the guinea-worm break on her slow crawl to freedom, that part of her which remains in the body will seek a new escape and form a new abscess, so it is the custom to reel her upon a twig or bit of straw, three or four turns per day. Sometimes

\* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

† Published by kind permission of Colonel L. A. HARWOOD, T.D., A.M.S., and Lieut.-Colonel T. C. HUNT, R.A.M.C.

Imperial Chemical Industries, Ltd., Hexagon House, Blackley, Manchester, kindly supplied the phenothiazine. Q.M.S. SHUTTLEWORTH, R.A.M.C., developed the emulsion.

she is two yards long, so the sufferer has a long time to howl! Severe cases leave permanent bone injury and a bad scar. There were, and may still be, villages where guinea-worms were so common an affliction that everybody had them every year."

The great necessity for an improvement in treating dracontiasis by the simple method mentioned in CAILLIE'S biography is obvious.

#### GEOGRAPHICAL DISTRIBUTION AND INCIDENCE.

These parasites are found in vast areas of Africa, India, Persia, Turkestan and Brazil. The infestation is endemic or hyperendemic in certain areas of these countries. In some parts it has a seasonal incidence depending on the necessity for towns and villages having to make communal use of water holes, step wells and ponds at the end of the dry season, either for bathing or washing purposes.

Few figures are available for estimating the incidence of dracontiasis in various areas. In the Chitaldrug district of Mysore (India), the annual incidence is put at 1,363 cases, which costs the Government in this area 40,000 rupees a year (MOORTHY, 1932). In Northern Nigeria, RAMSAY, using an intradermal antigen test, found that of 1,267 persons tested, 47.6 per cent. gave an immediate positive reaction. He estimated that 85 per cent. of visibly infected cases gave an immediate positive reaction. LINDBERG (1936) estimates that the population of Lai (Iran) is 8,000 to 13,000 persons and that 1,000 to 5,000 persons suffer yearly from dracontiasis. The Sudan Medical Reports of 1936 showed that 647 in-patients and 3,315 in-patients and out-patients of a hospital suffered from dracontiasis. In the Uganda area 108 cases of dracontiasis were treated in hospital in 1938.

#### PATHOLOGY.

Experimental evidence in animals suggests that the ingested larvae perforate the gut and develop in the retroperitoneal tissues. When the larvae have grown to adult size, copulation occurs and the impregnated female worm makes her way along the fascial planes to that part of the body where her instincts tell her water may be found in contact with the skin. *e.g.*, the feet, legs, hands or back. It has been estimated that this journey takes approximately a year to complete. Prior to its emergence under the skin, the worm may therefore be found lying relatively deeply in the tissues or more superficially. The worm may die before piercing the skin and give rise to sterile abscesses. Failure to absorb such dead worms may later give rise to the formation of bands of dense fibrous tissue which may become calcified. The worm may facilitate her passage by secreting an autolysing substance but recent zoological studies suggest that some *dracunculi* are capable of resisting extraction by the extrusion of a lateral spicule near the tail. There is nothing of diagnostic value in the blood picture.

## DIAGNOSIS.

A patient presenting with a bullous blister or sinuses on the feet or legs in an area where dracontiasis is endemic, is almost certainly suffering from dracontiasis. If this stage has not been reached, the sinuous shape of the worm under the skin should be looked for using reflected light and having previously sprayed the area with ethyl chloride. If the patient has had a previous infestation he can usually indicate the position of the worm in the tissues. Intradermal tests are rarely necessary. Worms presenting in atypical regions such as the thighs, hips, scrotum, hands, forearms and chest may cause some diagnostic confusion.

If the worm lies deeply the whole gamut of causes for both inflammatory and non-inflammatory thickening of the tissues will have to be considered. Many such cases are diagnosed as carbuncles, deep cellulitis, gumma, onchocerciasis, acute focal myositis, rheumatism, sciatica and focal periostitis. Sometimes an X-ray may show the calcified tract of a worm. In such cases the patient's opinion is often invaluable.

## CLINICAL TYPES.

On a pathological basis, the clinical types presenting themselves can be divided into six groups.

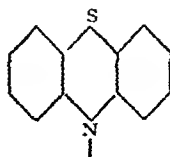
1. Worm in tissues, no sinus, blister formation or visual tract. Non-inflammatory.
2. As in 1, only inflammatory.
3. Blister, sinus formation, visual tract or worm presenting at sinus. Non-inflammatory.
4. As 3, only infected.
5. Residual types with fibrotic infiltration of tissues, such as skin, muscles, tendons or joints. Mechanical defects in associated structures.
6. As in 5, only no interference with the mechanism or locomotion.

## TREATMENT.

There are few diseases of such widespread incidence for which physicians or surgeons have felt less inclined to attempt any active treatment. Some treatments attempted only aggravate the condition. If the worm has not been secured many have plunged with surgical abandon into infected tissues in an attempt to dig out the offender. In most cases this is a case of looking for the proverbial needle in a haystack and serious infective sequelae may follow. The ideal treatment for this disease would be to introduce a drug into the tissues which would kill the worms, thus either facilitating their extraction or else leading to their rapid dissolution. Phenothiazine has this action.

## PHENOTHIAZINE.

Phenothiazine is a pale lemon yellow, photosensitive, crystalline powder; it is almost insoluble in water and has a faint but bitter taste. Its chemical formula is



It has been used with success both in the human and veterinary fields of helminthology. The author has recently used it in treating the multiple helminthic intestinal infestations of West African natives with satisfactory results. (Results to be published). DE EDS, STOCKTON and THOMAS (1939), reported that small daily doses of 2 to 3 grammes phenothiazine by mouth may in exceptional circumstances produce secondary anaemia. HUBBLE (1941) has reported anaemia and toxic hepatitis with jaundice in children following the oral treatment of thread worms with phenothiazine. This result is a possibility in view of the benzene structure of the phenothiazine molecule which brings it into chemical relationship with trinitrotoluene, dinitrobenzene, dinitrophenol and arsenobenzene derivatives, all of which are poisonous to both the liver and blood elements. In view of these findings a close watch should be kept on the blood picture, icteric index, and conjunctivae. None of the twenty-three cases in this series treated with phenothiazine developed toxic signs or symptoms. As much as 8 grammes intramuscularly (2 gm. at weekly intervals) has been given to patients without toxicity.

*Preparation of Phenothiazine for Injection.*—Insolubility of the drug in watery solutions made injections difficult. At first incisions were made into the thigh and the resulting wounds packed with the powdered drug. Little of the drug was absorbed into the circulation, owing to surface insolubility. Sterilized whole tablets of phenothiazine were next inserted under the fascia lata of the thigh. The results were promising but the technique labour consuming. Experiments were then started to produce an oily emulsion and the following method of preparation was adopted.

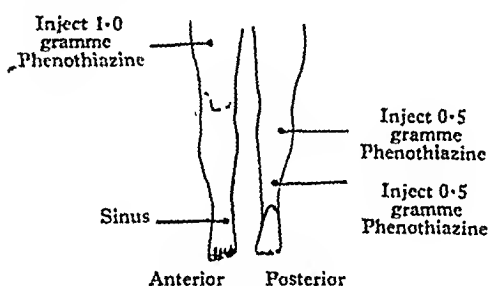
The phenothiazine finely powdered is triturated in a sterile mortar with 0.35 gramme of adeps lanae and about 15 c.c. of olive-oil, previously sterilized by heating at 150° C. for 1 hour. Five c.c. of sterile water is then added and the emulsion is produced. A further 20 c.c. of olive oil is then added and the emulsion is poured into previously sterilized 2 oz. medicine bottles and sealed. Sterilization was completed by heating the bottles in an autoclave at 115° C. for 30 minutes.

*Apparatus for Injection.*—One-20 c.c. and one-2 c.c. hypodermic syringes: twelve small hypodermic needles: six Bristol pattern transfusion needles with

adaptors ; one spirit-heated water sterilizer, table pattern ; two porcelain bowls, 2 to 3 in. diameter and of same depth ; one bottle 3 per cent. novutox ; methylated spirit and absolute alcohol ; swabs.

*Technique of Injection.*—This must be followed very carefully otherwise the phenothiazine will be thrown into clumps and block the needles.

The bottles of phenothiazine are placed upright in the sterilizer together with the syringes, needles and porcelain pots. Enough water should be added to cover the lower half of the bottles. The pots should be allowed to float in the water and should be spirit-fired with a few drops of absolute alcohol before use. The water is brought to the boil and the implements left in for half an hour. The affected limb is first injected with 3 per cent. novutox solution as follows. Suppose there is a guinea-worm sinus on the dorsum of the left foot with palpable tender induration of the associated calf muscles, 2 c.c. of novutox is injected into the vastus medialis muscle of the left thigh, near its centre and slightly to the outer side. The needle is left in to mark the position



SITES FOR INJECTING GUINEA WORM SINUS OF DORSUM OF RIGHT FOOT WITH INDURATION OF RIGHT CALF MUSCLES.

of infiltration. Two similar injections are made, one into the calf muscles in the upper third of the left leg, another into the dorsum of the lower third of the left leg, avoiding the tendo Achilles. Now take a bottle of phenothiazine emulsion out of the boiling water in the sterilizer and shake vigorously for two minutes. The emulsion is then poured into a dry spirit-fired pot and 20 c.c. drawn up into the 20 c.c. syringe. The small hypodermic needle is then removed from the thigh and the emulsion injected along its track using a Bristol transfusion needle for the purpose. Care must be taken to see that the emulsion is not sufficiently hot to burn the patient. The dorsum of the hand may be used as a suitable thermometer. If the injection is attempted with the emulsion at too low a temperature, the phenothiazine will precipitate and block the needles. The remaining 20 c.c. of emulsion in the pot is then drawn into the syringe and 10 c.c. injected into each of the other two sites in a similar manner. The sites of injection should be firmly massaged afterwards for five minutes. There may be some slight discomfort at the sites of injection for a few hours.

The most important part of the whole treatment is to see that half of the emulsion is injected as near the course of the buried worm as possible. Both local and general concentration of the drug are very important. Without local

area will lead to dissolution of the induration and drying up of the sinuses in 10 to 14 days. It is very important to train the nursing staff who look after these cases, in the methods of extraction. Attempts to roll up the worm on a stick or to press it out of a sinus should first be preceded by strong pressure with the fingers along the course of the worm tract in the direction of the sinus. This milking action should be done for about 2 minutes. In this way greater lengths of worm may be extracted. After attempts at extraction, the stick should be fixed to the skin by adhesive strapping and a dry sterile dressing applied. The diagnosis and treatment of coincidental diseases is of great importance.

#### SUMMARY.

A survey of twenty-three cases of dracontiasis treated by phenothiazine has been made. The incidence, pathology, diagnosis and clinical types met with are discussed.

Great importance should be attached to the methods of preparing the drug for injection and to the actual technique of injection. The most important part of the technique is to inject at least 1 gramme of phenothiazine into the vicinity of the buried worm.

The introduction of phenothiazine therapy has led to a remarkable reduction in the amount of invalidism from this disease. The hospitalization rate has been reduced but could be further reduced by earlier diagnosis.

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APPENDIX.  
SUMMARY OF CASE PROTOCOLS.

Case.	Number.	Month.	Race.	Number and Location of Sinuses.	Tissue Induration	Infected.	Worms Presenting.	Worms out.	Treatment.	Days under Treatment.	Days in Hospital.	Result.
1	35892	Sept.	N.	1 Left ankle joint - " foot	- +	+	-	-	P.2	5	16	++ Relapse 4 weeks later
2	35966	Aug.	N.	2 Right leg 1 Right leg	+	+	-	-	P.1	20	31	+ Relapse 8 weeks later
3	35957	"	N.	1 " knee joint 1 " ankle "	+	+	-	-	P.1 and P.2	17	23	+
4	44117	"	N.	1 Left leg 1 " ankle joint	- +	+	-	-	P.1	11	21	+
5	33359	Sept.	N.	1 " leg 1 " ankle joint	+	+	-	-	P.2	13	17	+
6	44005	Aug.	N.	2 Right thigh " leg	- +	+	-	-	P.1 and P.2	18	22	+
7	33355	Sept.	N.	3 " "	+	+	-	-	P.1 " P.2	10	22	+
8	44036	"	N.	1 " foot	-	+	-	-	P.1 " P.2	21	37	++
9	44098	"	N.	1 Left ankle joint 1 Right "	- -	+	-	-	P.2	14	17	+
10	44104	"	N.	2 " "	-	+	-	-	P.2	22	26	++
11	37009	Aug.	N.	1 Left - Right foot	+	+	+	-	P.2	20	33	++
12	34042	Sept.	N.	1 Left ankle joint - " leg - " foot	+	+	-	-	P.3	12	14	+
13	35968	Oct.	SL.	2 Right ankle joint	+	+	+	2	P.3	23	25	++
14	42967	"	N.	1 Left - " leg	+	++	-	1	P.3	6	7	++
15	44065	June	N.	1 Right calf 1 " ankle joint	+	+	-	1	Extraction	10	10	O
16	44112	"	N.	1 Left leg 1 Right calf	+	+	+	2	"	78	78	
17	43425	July	N.	1 Left ankle joint - " leg	- +	+	+	1	"	15	17	++
18	35954	"	N.	1 Right ankle joint 1 Left knee "	- -	+	-	-	Attempted extraction	31	35	O
19	40446	Aug.	N.	1 " " " 2 " leg 2 Right ankle joint 1 Left foot	- + - -	+	-	-	Extraction	17	17	B
20	50611	"	N.	1 " " " 1 " hand	- -	+	+	1	"	17	17	+
21	43042	July	N.	1 " ankle joint 1 " thigh 1 " knee joint	+	+	-	-	Attempted extraction	13	13	B



Case.	Number.	Month.	Race.	Number and Location of Sinuses.	Tissue Induration	Infected.	Worms Presenting.	Worms out.	Treatment.	Days under Treatment.	Days in Hospital.	Result.
22	33957	Aug.	N.	1 Right ankle joint 1 " knee "	-	+	-	-	P.1 + P.2	22	23	+
23	44031	"	N.	1 " foot 1 Left ankle joint 1 Right knee "	-	+	-	1	Extraction	24	24	B
24	40542	July	N.	1 Left " " 1 " ankle " 1 right calf 1 Left elbow	-	+	-	1	"	13	13	B
25	44413	"	N.	1 " ankle joint - " foot	+	+	-	1	Incision and injection of biniodide of mercury	38	44	+
26	36440	"	N.	1 " ankle joint	+	+	+	-	Attempted extraction	48	48	O
27	31571	Aug.	SL.	- Right	-	-	-	-	1 Extraction	2	8	++
28	62014	"	SL.	2 " knee joint - " ankle " - Left " "	+	+	+	1	"	31	31	O
29	31928	"	N.	1 " leg	+	+	+	1	"	9	9	++
30	43422	"	N.	1 Right ankle joint - Left leg	+	+	-	-	Rest. At- tempted ex- traction	34	34	O
31	37507	July	N.	1 " ankle joint 1 Right "	+	+	-	-	Rest	22	22	B
32	41329	"	N.	- " calf	+	+	-	-	"	26	26	B
33	35966	Oct.	N.	1 " ankle joint 1 " leg 1 Left thigh 1 " hip - Right calf	+	+	-	-	P.3	35	35	++
34	34411	Aug.	N.	1 Left leg	+	+	-	-	Attempted extraction	33	33	+
35	—	"	N.	2 Right " 1 Left calf - Right knee joint	+	+	-	-	Attempted extraction	29	29	B
36	31567	"	N.	1 " big toe - " leg	+	+	-	1	Extraction	28	28	+
37	35968	July	N.	- " foot	+	+	-	1	"	11	11	++ Relapsed 2 months later
38	43388	"	N.	Left buttock	+	+	-	1	"	12	12	+
39	35904	"	N.	1 Right ankle joint - " thigh - Left calf	+	+	-	1	"	54	54	++ Relapsed 3 months later

## APPENDIX—(Continued).

Case.	Number.	Month.	Race.	Number and Location of Sinuses.	Tissue Induration.	Infected.	Worms Presenting.	Worms out.	Treatment.	Days under Treatment.	Days in Hospital.	Result.
40	44053	July	N.	1 Left ankle joint 1 Right ..	— +	+	—	1	Extraction	78	78	+
41	37245	"	N.	1 Left ..	+	+	—	1	"	15	15	+
42	43402	"	N.	1 ..	+	+	+	2	"	25	25	+
43	37507	June	N.	1 .. foot — Right calf	— +	+	—	1	"	25	25	++
44	43399	"	N.	1 Left ankle joint	+	+	—	—	Attempted extraction	27	27	O
45	48187	"	N.	1 .. calf 1 .. calf	— +	+	—	1	Incision, sulphonamide. Extraction	37	37	+
46	35754	"	N.	1 Right leg	+	+	—	1	Incision. Extraction	28	28	++
47	40542	July	N.	1 .. knee joint 1 Left leg	— +	+	—	1	Extraction	16	16	+
48	44065	"	N.	3 Right knee joint 1 .. ankle ..	— —	+	—	3	"	34	34	O
49	357541	June	N.	1 .. leg 1 .. ankle joint	+	+	—	—	Incision attempted. Extraction	46	46	+
50	43415	"	N.	1 Left leg	+	+	+	—	Elastoplast	77	77	B
51	37507	"	N.	1 .. foot — Right calf	— +	+	—	—	Extraction	25	25	Relapsed 1 month later
52	—	July	N.	1 Left ankle	+	+	—	—	Attempted extraction	25	25	O
53	48025	Nov.	N.	1 Right leg	+	+	+	—	P.3	5	11	++
54	35892	Oct.	N.	2 .. knee joint	+	+	—	—	P.3	7	18	++
55	35904	"	N.	1 Left thigh — Right hand	— +	+	—	1	P.3	8	18	++
56	44013	"	N.	1 Left leg	+	+	—	—	P.3	10	20	++
57	48888	"	N.	1 Right ..	+	+	—	1	T Foments P.3	15	35	+
58	51698	Nov.	N.	2 ..	+	+	—	—	P.3	10	13	++
59	44017	"	N.	1 Scrotum	+	+	—	1	P.3	6	46	++

## EXPLANATION OF TERMS.

- N. = Nigerian. SL. = Sierra Leone.  
 P.1 = Packing incised wounds with powdered phenothiazine.  
 P.2 = Inserting tablets of phenothiazine under fascia lata.  
 P.3 = Intramuscular injection of phenothiazine.  
 ++ = Fit for full duty; all sinuses dry; no inflammation.  
 + = Fit for full duty; no inflammation; slight discharge.  
 O = Unfit for duty; sinuses discharging: Inflamed.  
 B = Boarded out of Army.



## DURAND'S DISEASE: A VIRUS INFECTION TRANSMISSIBLE TO ANIMALS AND MAN.

BY

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In 1940 DURAND described a new virus which he had isolated from his own blood. The virus, which was provisionally called virus D, was found to be pathogenic for guineapigs and for certain other laboratory animals. During a visit to the Pasteur Institute of Tunis in March and April, 1940, Dr. PAUL DURAND very kindly placed the new virus at my disposal. The study of its characteristics here recorded in the main confirms those described by DURAND, although certain new facts have come to light.

### PATHOGENICITY FOR MAN.

The illness, in the course of which the virus was first isolated, occurred in June, 1939, beginning with a feeling of lassitude, loss of appetite, a temperature of 100·6° F., insomnia alternating with bizarre dreams and erratic muscular pains. The principal symptoms throughout the illness were complete anorexia, constipation and insomnia. The temperature, which gradually returned to normal by lysis on the 11th day, was for the most part between 102° F. and 103·8° F., the latter, the highest recorded, occurring on the 5th day of illness. During the course of the disease there was a loss of body weight of between 7 and 8 kg. The blood count on the 3rd day showed 58 per cent. polymorphonuclear leucocytes, 1 per cent. eosinophils, 3 per cent. large mononuclears, 12 per cent. large lymphocytes and 26 per cent. small lymphocytes. Blood cultures were bacteriologically sterile: there was no agglutination of typhoid or paratyphoid organisms and no positive Weil-Felix reaction. The patient had had murine typhus 5 years previously. Blood removed from a vein on the 2nd day of fever, when injected into a guineapig, caused a febrile reaction. The disease could be passaged in series in guineapigs.

The pathogenicity of the virus was further shown by DURAND by the infection of two patients who required pyrotherapy with an emulsion of infected guineapig spleen. The first patient had a reaction with fever (102·8° F.) on the morning of the 4th day following the injection. Fever continued for 6 days. Apart from slight headache at the beginning and constipation there were no particular symptoms. Blood taken on the 1st day was virulent for guineapigs. The urine was not infective.

The second patient, 48 hours after inoculation, had shivering attacks and

a slight rise in temperature. On the following day the temperature rose to 103.6° F. with a pulse of 108 and post-ocular headache. A slight cough was present; the temperature on the 8th day after inoculation was normal. On the 7th day, although no nervous symptoms were present, a lumbar puncture was performed; it showed 65 cells per c.mm., 98 per cent. being polymorphonuclear leucocytes, with 0.25 gramme albumin and a normal sugar content. The leucocytes in the blood showed a slight polymorphonuclear leucocytosis, rapidly followed by a leucopenia and relative lymphocytosis. The blood, by inoculation into guineapigs, was shown to contain virus from the 4th to the 21st days; urine from the 5th to the 17th days was also infective for guineapigs. Cerebrospinal fluid taken on the 6th day after injection infected one of two guineapigs.

#### A LABORATORY INFECTION.

The pathogenicity of the virus for man is also shown by the following laboratory infection: G.M.F., laboratory worker, aged 47; previous medical history, typhoid in 1917; entire absence of any symptoms of indigestion or gastric discomfort; 5 weeks before infection had suffered from a mild attack of murine typhus with 5 days of fever; patient had returned to work in the laboratory 18 days before the present illness. The exact incubation period cannot be determined though it was certainly less than 8 days and probably not more than 4.

On 29.5.40 patient felt very tired and on the following day developed nasal catarrh with considerable running from the nose and an irritating dry cough without sputum. There was a feeling of lethargy with aching in the legs. In the evening the temperature was 99.2° F. On 2.6.40 the temperature had risen to 100° F., the cough continued, but the nasal discharge had decreased; there was slight post-orbital pain and vague muscular pains in the legs and thighs: the feeling of tiredness continued and the temperature gradually rose till on the evening of 6.6.40 the temperature was 102.6° F. (Chart 1). No

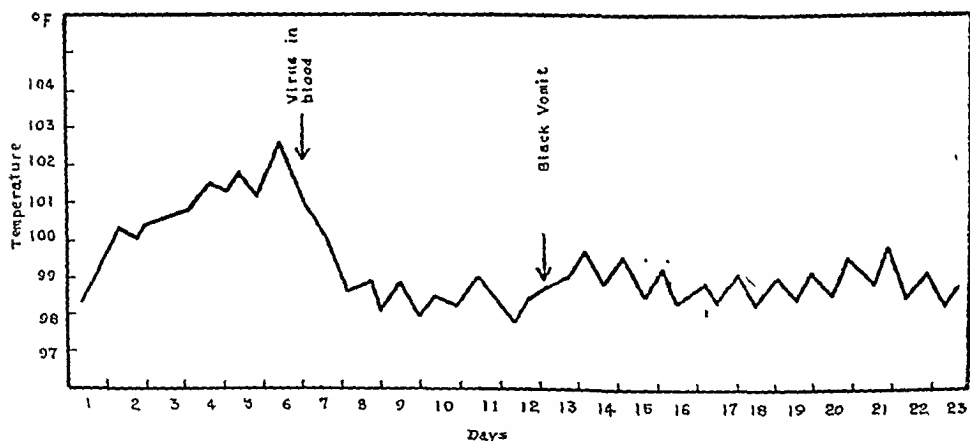


CHART 1. Temperature chart of G. M. F. suffering from virus D infection.

definite localizing symptoms were present during this period; the conjunctivae were not congested; the tongue was covered with a whitish fur down the centre. There was no sore throat. The heart and lungs were normal. The pulse was in conformity with the temperature. The splenic dullness was slightly increased but the spleen was not palpable. The liver was not enlarged, and the urine, though deep yellow in colour, was

not decreased in amount; there was no albumin or sugar, the dry cough lasted for 4 days; insomnia, in spite of the feeling of fatigue, was a noticeable feature; the bowels were extremely constipated. Blood taken on 7.6.40 showed Hb., 92 per cent.; leucocytes, 7,200 with normal differential count with the exception of a number of immature polymorphonuclear leucocytes.

The temperature fell practically to normal on 8.6.40 and remained low till 12.6.40, on which day the patient came downstairs for the first time. There was a considerable feeling of weakness and in the evening quite suddenly a severe headache developed, beginning in the occipital region, passing over the right temporal region, and localizing in the left frontal region. This sudden and severe headache was associated with a feeling of collapse and slight nausea. The temperature was subnormal (96.4° F.). Aspirin, 5 grains, was taken in an effort, which was unavailing, to relieve the headache.

Early on the following morning, 13.6.40, about 2.30 a.m., the patient woke up with a feeling of nausea and after considerable retching vomited about 20 c.c. of brownish fluid. At intervals of about an hour vomiting continued till 7 a.m., the vomited material being typically coffee-ground in appearance; about 500 c.c. was vomited in all. There was a feeling of intense collapse, but no further vomiting occurred. A severe headache, however, remained localized over the left frontal region and did not completely disappear for more than 10 days. At the same time slight fever returned in the evening and continued for 8 days. There were also occasional neuralgic pains at the back of the neck, over the lower part of the thorax and in the muscles of the calf. There was no abnormal tenderness in the abdomen. Constipation and insomnia were still present.

The temperature returned to normal on 19.6.40, but on the evenings of 22.6.40 and 23.6.40 it again rose to 99.2° F. and 99.4° F., with increase in the feeling of tiredness. Convalescence was slow and was associated for about a fortnight with neuralgic pains in the calf muscles on walking. During the illness there was loss of nearly 14 lbs. in weight.

An X-ray examination during convalescence showed no evidence of any ulceration in the stomach or duodenum. Strength was not completely restored for nearly 2 months after the beginning of the illness.

Blood taken from G.M.F. 30.5.40 was inoculated intraperitoneally into two guineapigs. One guineapig showed an elevation of temperature to 104° F. on the 9th day and 105° on the 10th day, when it was killed. The G.M.F. strain was isolated from the spleen of this guineapig. The second of the original guineapigs showed a single elevation of temperature to 104.6° F. on the 14th day following inoculation and later showed immunity to inoculation with the G.M.F. strain. Complete cross immunity between the G.M.F. and stock strain of D virus was demonstrable in convalescent guineapigs.

### THE EXPERIMENTAL DISEASE IN THE GUINEAPIG.

The guineapig appears to be the most suitable laboratory animal for the investigation of virus D since it reacts with fever and with characteristic lesions. The guineapig can be infected by intradermal, subcutaneous, intraperitoneal, intracerebral and intratesticular inoculation. The virus will also infect if placed on the lightly scarified skin or cornea, while in three out of eight attempts fever has followed the application of a suspension of virus to the apparently normal skin after the hairs have been clipped. Intranasal instillation of the virus also leads to a generalized infection. Routine passage of the virus is carried out with a 10 per cent. suspension of infected guineapig spleen in physiological saline: the injections may be made either subcutaneously or intraperitoneally. The incubation period with this dose of virus varies from 2 to 5 days but the majority of animals react in from 2 to 3 days. With smaller doses of virus the incubation may be as long as 10 to 14 days.

The temperature rises to a maximum of between 105° and 106° F. on the 2nd or 3rd day of fever, remains high for from 4 to 8 days, and then falls by lysis in those animals that recover. In those guineapigs that die there is a sudden and dramatic fall in temperature just before death (Chart 2); however,

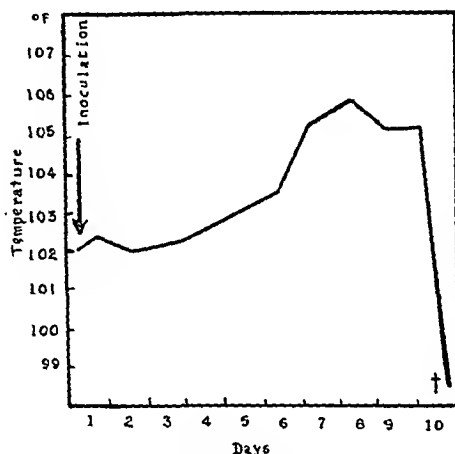


CHART 2. Temperature chart of guineapig injected subcutaneously with virus D: fatal result.

only about 10 per cent. of the guineapigs inoculated die; all, and more especially those that die, exhibit some loss of weight. After intraperitoneal injection this loss of weight and the fever are the only symptoms observable during life; after subcutaneous inoculation into the groin the lymph node, on the affected side, becomes enlarged and the surrounding tissues swollen, and in a few cases there has been considerable oedema in the subcutaneous tissues on the affected side.

On examination at death there is loss of subcutaneous fat, while in the groin the indurated area is haemorrhagic; the inguinal lymph node on the infected side is about three times the size of that on the opposite side and is pinker in colour. In some guineapigs the subcutaneous tissues show a gelatinous oedema which occasionally extended as far forwards on the infected side as the thorax. Loss of fat also is noted in the abdomen while the retroperitoneal lymph nodes are swollen and reddened, more especially on the inoculated side. The spleen is always considerably enlarged, dark red in colour, and very friable. The liver is brownish-yellow in colour, and the adrenals, kidneys and other abdominal organs show no gross abnormality. The condition of the lungs is variable. In some guineapigs there is little departure from the normal, in others the lower lobes alone are consolidated while in others the pneumonic process extends throughout both lungs. The brain exhibits no macroscopic change. The characteristic gross changes in the spleen, liver and lungs are present in animals inoculated intracerebrally and intraperitoneally as well as by intranasal instillation. After intratesticular

inoculation the injected organ is slightly reddened and swollen while tiny haemorrhagic areas are present in the tissues surrounding the vas deferens.

Ten intratesticular passages were carried out: after the third passage the incubation period became reduced from 48 to 36 hours and finally, on the eighth to ninth passages, to 12 to 24 hours. After intracerebral inoculation the period from inoculation to the development of fever was on the average 7 days, while application of infected material to the lightly scarified skin caused a prolongation of the incubation period to from 10 to 15 days.

Total and differential blood counts in guineapigs showed no abnormality in the number or character of the red cells; the leucocytes showed a slight reduction in number affecting more especially the polymorphonuclear leucocytes; the large mononuclear leucocytes were increased from 3 to 4 per cent. to from 10 to 17 per cent.

Aerobic and anaerobic cultures of the blood and spleens of infected guineapigs were as a rule sterile; occasionally, however, a small Gram-negative bacillus was isolated from the blood.

#### THE INFECTIVITY OF THE VIRUS FOR OTHER ANIMALS

In addition to guineapigs the following animals have been inoculated: the rhesus monkey *Macaca mulatta*, European hedgehog *Erinaceus europaeus*, cat, dog, ferret, golden hamster *Cricetus auratus*, rabbit, rat, mouse, Orkney vole *Microtus orcadensis*, pigeon, and canary. No local reaction or febrile reaction followed subcutaneous injection in any animal. Of two Orkney voles injected, one, accidentally killed 4 days later, was found to have virus in the spleen, while the second, which died 9 days after injection, also had virus in the spleen. The spleen was slightly enlarged. Ferrets, in addition to being injected subcutaneously, were inoculated intracerebrally with 0.06 c.c. of a 10 per cent. suspension of infected guineapig spleen. Two ferrets were anaesthetized with ether and a similar suspension was instilled into the nares. No febrile reactions occurred in these animals. Mice, in addition to being inoculated subcutaneously, were injected intracerebrally and intraperitoneally, while infected material was instilled intranasally when the animals were under ether anaesthesia. None of the inoculated mice died but after intraperitoneal injection it was found as shown by inoculation into guineapigs that virus was present in the mouse spleen for at least 42 days after injection. No constant enlargement of the spleen was noted in mice. It is possible to carry out successive passages in mice by intraperitoneal injection. Two mice were inoculated intraperitoneally with 0.25 c.c. of a suspension of infected guineapig spleen diluted  $10^{-4}$ ; at an interval of a week the mice were killed and their spleens ground up in physiological saline in a dilution  $10^{-4}$  and injected into two other mice. Six successive passages were thus made by intraperitoneal inoculation into mice, the virus concentration in the last mouse being estimated by the intraperitoneal injection of 1 c.c. of decimal dilutions of mouse spleen into guineapigs. Virus was present up to a dilution of  $10^{-6}$ . Ten successive intracerebral passages



were made in mice: no nervous symptoms developed and virus could be found in the mouse spleens. After intranasal instillation in mice lung lesions were seen in the form of scattered areas of congestion and again virus was present in the spleen. D virus thus causes an almost inapparent infection in mice. The same is true of rats. After intraperitoneal injection the virus could be detected in the spleen of rats by intraperitoneal inoculation of guineapigs for 35 days.

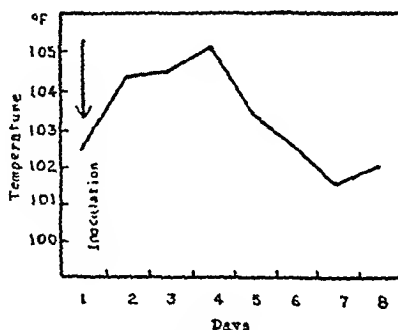


CHART 3. Temperature chart of rhesus monkey inoculated intracerebrally with virus D: recovery.

Two rhesus monkeys inoculated subcutaneously in the right groin exhibited no significant rise in temperature. After intracerebral injection of 1 c.c. of a 10 per cent. suspension of infected guineapig spleen into two other rhesus monkeys a febrile reaction occurred after an interval of 48 hours (*cf.* Chart 3). One of these rhesus monkeys developed weakness of the hind legs with loss of appetite and ruffling of the fur. Virus was present in the blood of the monkey

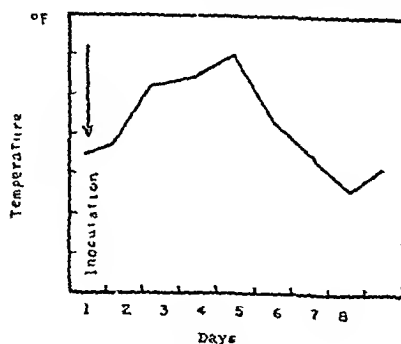


CHART 4. Temperature chart of cat inoculated intracerebrally with virus D: recovery. on the second day after inoculation and when killed was isolated from the blood, brain, spleen and lung. The lungs were congested at the base and the brain was congested; otherwise there were no naked eye changes. The spleen was not enlarged.

Two cats inoculated subcutaneously showed no febrile reaction but two

other cats, injected intracerebrally with 0.03 c.c. of a 10 per cent. suspension of infected guineapig spleen, showed a febrile reaction (Chart 4). One cat, killed on the 8th day after inoculation, showed a few small areas of congestion throughout the lungs. Virus was present in the blood, brain, spleen and liver. A fifth cat was inoculated intracerebrally with a 10 per cent. suspension of the spleen of Cat 4; a febrile reaction occurred on the 3rd day after inoculation. Virus was present in the circulating blood at the beginning and end of the febrile period, which lasted 5 days.

The results in dogs were very similar to those in cats. Subcutaneous injection caused no febrile or local reaction. Of two dogs injected intracerebrally, one showed a febrile reaction 3 days after injection but no nervous symptoms; the second showed a rise of temperature to 104.4° F. on the 2nd day after inoculation. This rise of temperature lasted for only 24 hours. On

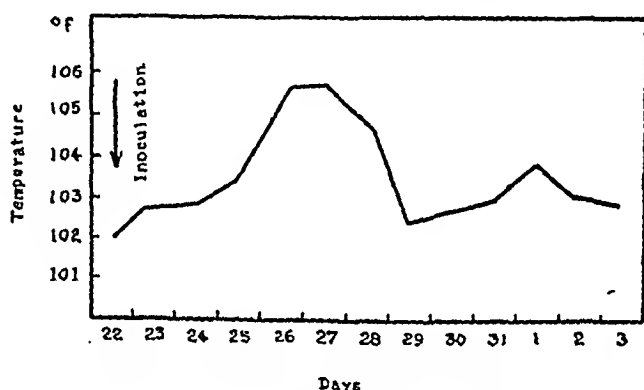


CHART 5. Temperature chart of dog inoculated intracerebrally with virus D: recovery.

the 20th day after inoculation this dog began to develop nervous symptoms, snapping and whining. There was no rise in temperature, but muscular weakness developed rapidly and the eyelids became matted together with exudate. The dog was killed on the 23rd day after inoculation. At death bronchopneumonic changes were present in both lungs. Virus was present in the brain, blood and spleen.

DURAND noted that intracerebral inoculation of a rhesus monkey was followed by paresis of the hind legs and death. Conjunctivitis and nervous symptoms were also noted in dogs.

Rabbits injected intraperitoneally and subcutaneously showed no characteristic temperature response. Two rabbits inoculated intracerebrally with 0.2 c.c. of a 20 per cent. suspension of guineapig spleen showed fever in 48 hours and slight evidence of conjunctivitis.

Golden hamsters inoculated intraperitoneally with 2 c.c. of a 20 per cent. suspension of infected guineapig spleen showed irregular fever during the next month. In two hamsters examined 30 days later the spleens were enlarged and both contained virus.

DURAND also infected the merion (*Meriones shawi*), a small north African rodent, and noted fever with a local reaction after subcutaneous injection. Fever also followed intraperitoneal injection. No nervous symptoms occurred after intracerebral inoculation.

#### HISTOLOGICAL CHANGES.

The pathological changes noted were most marked in the guineapig. These are therefore first described.

Tissues from infected guineapigs were fixed in Zenker's fluid and sections were stained with Heidenhain's iron haematoxylin and eosin and with Giemsa's stain. In addition, smears from all organs were stained by Giemsa's method in order to determine the presence of elementary bodies. No such elementary bodies could be found.

When infected guineapig spleen is injected subcutaneously into the groin the loose fatty tissues become heavily infiltrated with cells, consisting for the most part of polymorphonuclear leucocytes with a smaller number of large mononuclear cells (Plate, Fig. 1). The blood vessels are dilated and congested, the endothelial cells swollen, and in many vessels a process of thrombus formation occurs, a number of polymorphonuclear leucocytes and mononuclear cells being present in the lumen of the vessels. Many of these capillaries rupture, giving rise to haemorrhages into the surrounding connective tissue. Infiltration of the muscles on the abdominal wall is also noted, the fibres being separated by invading cells. In the immediate neighbourhood of the inguinal lymph node the infiltration was extremely dense.

**Lymph Nodes.** The most striking changes were seen in the lymph nodes in the neighbourhood of the site of inoculation and consisted of a hyperplasia of the reticulo-endothelial cells, together with a decrease in the number of lymphoid cells (Plate, Fig. 2), both in the germinal follicles and in the sinusoids. Many of the large endothelial cells showed vacuolation of the nuclei (Plate, Fig. 3), a condition which has been previously noted in glandular fever. Phagocytosis of red blood corpuscles was occasionally seen but no inclusion bodies, either intranuclear or cytoplasmic, could be demonstrated. The blood vessels were congested but no actual haemorrhages were apparent. Occasional polymorphonuclear leucocytes were seen in the sinuses but there was no indication of the formation of stellate abscesses, such as occurs in lymph nodes infected with the virus of lymphogranuloma venereum. Eosinophil cells were not seen and giant cell formation was absent. The fibrous septa of the lymph nodes were less prominent than usual, probably as a result of the hyperplasia of the reticulo-endothelial cells lining the sinuses. Similar changes were present in the retroperitoneal lymph nodes.

**Spleen.** The histological changes were very similar to those described in the lymph nodes and consisted of hyperplasia of the reticulo-endothelial cells with, in some areas, almost complete obliteration of the germinal follicles (Plate, Fig. 4). In the spleens of some animals phagocytosis of red cells was very common, in others phagocytosis was not greatly increased. Vacuolation of the nuclei of the endothelial cells was well seen in all spleens examined. Polymorphonuclear leucocytes and a small amount of granular debris were occasionally seen.

**Liver.** The cells of the lobules showed considerable fatty infiltration except in the outer zones where the cells appeared quite normal. The Kupffer cells were swollen and prominent throughout the lobules, but only occasional infiltrating cells were found in the sinusoids, usually small round cells. The actual portal spaces appeared normal (Plate, Fig. 5).

The other abdominal organs, kidney, pancreas, stomach, and intestines appeared normal.

The **brain**, after subcutaneous or intraperitoneal injection of the virus, showed no abnormal changes but intracerebral injection of the virus was responsible for the presence of a few large mononuclear and epithelioid cells in the meninges, more especially at the base of the brain.

The **lungs** showed some degree of interstitial pneumonia more especially in the lower lobes. The earliest stages of the process consisted of swelling and proliferation of the

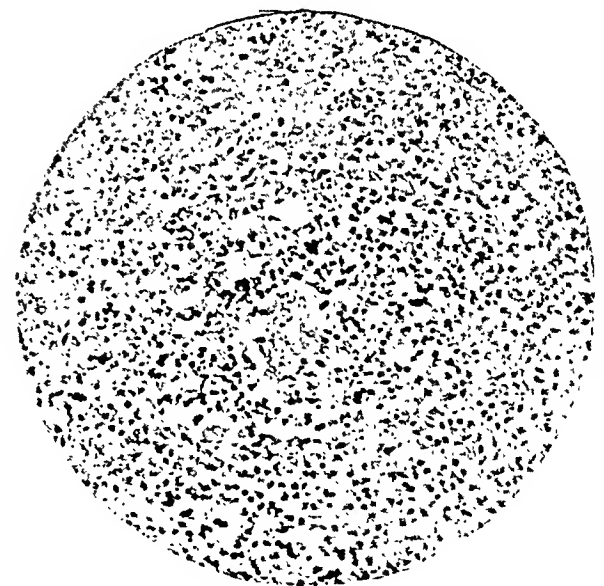
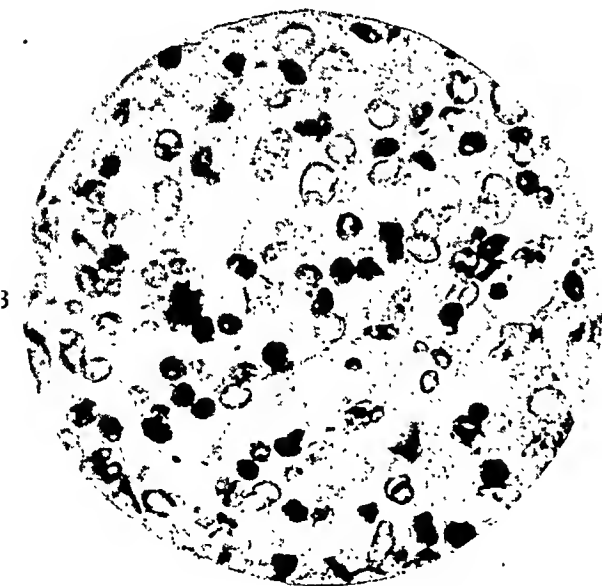
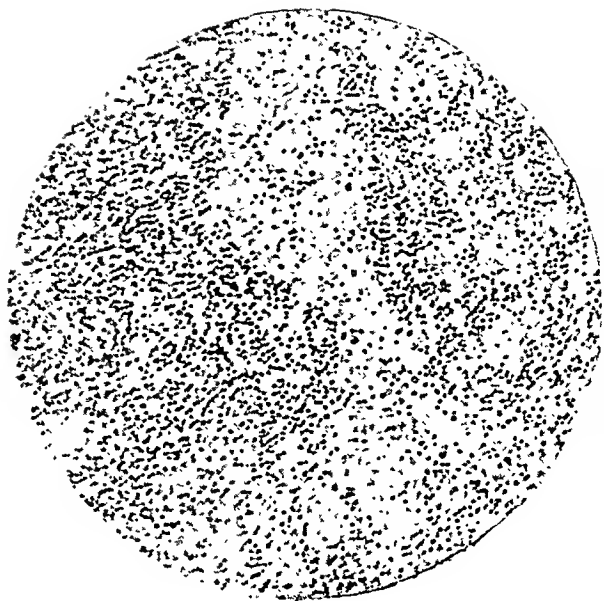
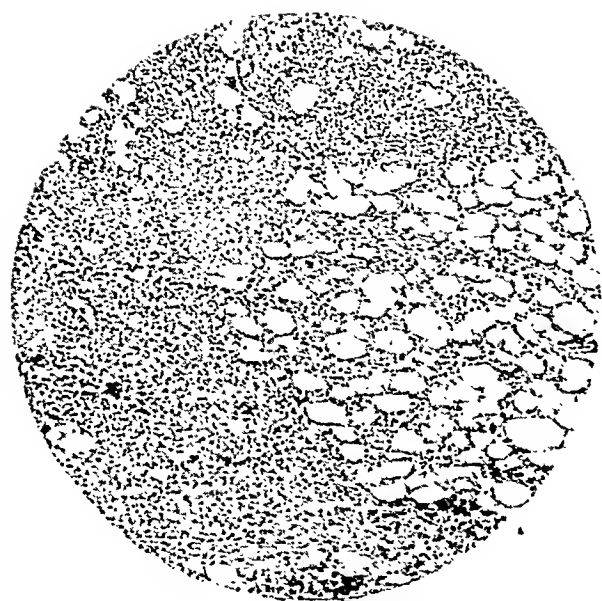


FIG. 1. Subcutaneous tissue from the groin of a guineapig injected subcutaneously in the right groin with virus D showing polymorphonuclear leucocytes and a few mononuclear cells with some haemorrhagic areas.  $\times 75$ .

FIG. 2. Lymph node from the groin of a guineapig injected subcutaneously with virus D showing hyperplasia of the reticulo-endothelial cells.  $\times 150$ .

FIG. 3. Lymph node showing vacuolization of the nuclei of endothelial cells.  $\times 750$ .

FIG. 4. Spleen from guineapig inoculated subcutaneously with virus D showing disappearance of lymphocytes from a germinal follicle.  $\times 175$ .

*All sections stained with Heidenhain's iron haematoxylin and eosin.*



reticulo-endothelial cells lining the alveoli and in their walls. At the same time small nodules of large endothelial cells were noted in the alveolar walls. Later, owing to the increased number of cells in the alveolar walls, certain of the alveoli were completely obliterated (Plate, Fig. 6). The bronchi appeared normal.

After subcutaneous or intraperitoneal inoculation of the virus the testicle appeared normal but after direct injection into the testicle an intense cellular reaction was seen in the interstitial tissue. The infiltrating cells consisted of large epithelioid and mononuclear cells, together with a few small lymphocytes (Plate, Fig. 7). The tunica vaginalis was also infiltrated with round cells (Plate, Fig. 8). No polymorphonuclear cells were present. The actual secretory tubules were normal. No cytoplasmic or intranuclear inclusions were noted either in the testicle or in the lungs.

In the stomachs of guineapigs that had died of the disease there was rarely any food but the mucosa was covered with flecks of brownish black material. In sections (Plate, Fig. 9) the capillaries on the surface of the stomach were very congested and in places appeared to have ruptured. This change is of interest in view of the occurrence of black vomit in a human case.

The histological changes met with in other animal species differed quantitatively but not qualitatively from those seen in the guineapig. In the spleen there was to be found in all species some hyperplasia of the reticulo-endothelial cells, while in the lungs, more especially of the dog, cat and monkey, interstitial changes were seen. In the brains of cat, dog and monkey the local lesions at the site of inoculation were very pronounced. In the cat brain there was a large focus (Plate, Fig. 10) made up of polymorphonuclear leucocytes and mononuclear cells (Plate, Fig. 11), while in areas adjacent to the local lesions perivascular infiltration was noticeable, small lymphocytes predominating. In the dog and monkey brain similar, though less striking lesions, were seen.

#### CHARACTERISTICS OF VIRUS D

The fact that virus D is widely distributed in the guineapig and in other experimental animals has already been described. Whole blood and serum were found to be infectious within 12 hours of subcutaneous inoculation and consistently up till the time when the temperature fell to normal. In one instance virus was isolated from a guineapig 7 days after the temperature had returned to normal. The virus is present in high concentration in the serum at the height of the fever; 1 c.c. of a  $10^{-12}$  dilution of blood produced a febrile reaction for 2 days on subcutaneous injection into a guineapig. The animal was subsequently immune to an injection of 1 c.c. of a  $10^{-1}$  suspension in saline of infected guineapig spleen. Virus was found to be present in the bile in small amounts on the three occasions on which tests were made; on the other hand, virus was isolated from the urine of only one out of three guineapigs killed during the height of the fever. It was absent from the faeces on the three occasions on which these were tested.

Virus continues to be present in the spleen of guineapigs after the fall of temperature as shown by the subcutaneous inoculation of a 1 in 10 suspension of infected spleen into fresh guineapigs, as shown in table below.

*Resistance to Temperature.* Virus D is comparatively resistant to inactivation by temperature. When infected guineapig spleen was kept in a Petri dish at room temperature (18 to 20° C.) it was active for 3 but not for 6 days. Under the same conditions at +4° C. in the ice chest virus in infected spleen was active for 18 days. A 10 per cent. suspension in saline kept in the ice

TABLE I.

Number of Days from Fall of Temperature to Removal of Spleen.	Result of Infectivity Test.	
	Positive.	Negative.
21	2	1
28	2	0
35	1	3
51	0	2
252	0	1

chest at  $+4^{\circ}\text{C}$ . in a tube covered with a rubber cap was still active after 4 months. At  $37^{\circ}\text{C}$ . in serum Tyrode the virus was active after 7 days, but the incubation period after the injection of 1 c.c. was 13 days; virus was destroyed after 14 days at  $37^{\circ}\text{C}$ . After the virus had been frozen and dried it was active after 6 and 11 but not after 18 months. The results of inactivation by heat are shown in Table II.

TABLE II.  
Inactivation by Heat.

Temperature.	Length of Time of Exposure. In Minutes.			
	20	30	40	60
$60^{\circ}\text{C}$ .	+	+	—	—
$65^{\circ}\text{C}$ .	+	—		

After being injected with infected guineapig spleen heated for 20 minutes at  $65^{\circ}\text{C}$ . a guineapig showed no febrile reaction but was subsequently immune to an injection of 1 c.c. of a 10 per cent. suspension of infected guineapig spleen.

*Preservation in Glycerine.* When kept in a mixture of equal parts of glycerine and saline the virus was present 8 months later but was inactivated after 16 months.

*Resistance to Chemicals* 1 in 1,000. A suspension of infected guineapig spleen in saline was treated with 1 per cent. crystal violet at  $37^{\circ}\text{C}$ . and then for 7 days in the ice chest. Virus was still present, although the incubation period was 9 days. Formalin in a concentration of 0.2 per cent. did not inactivate the same suspension of the virus kept at  $4^{\circ}\text{C}$ . in the ice chest for 2 days but after 10 days' contact no febrile reaction occurred in two test guineapigs. These guineapigs were not immune 14 days later.

*Filtration.* After centrifugation at 3,000 r.p.m. for 10 minutes the supernatant fluid from a suspension of infected guineapig spleen 1 in 100 in serum

broth was filtered through Berkefeld V, W and N candles and through an EK pad. The filtrates inoculated intraperitoneally into guineapigs in doses of 1 c.c. were infectious.

In order to determine the size of the virus when filtered through gradocol membranes 1 in 100 suspensions of infected guineapig spleen in serum broth were centrifuged at 3,000 r.p.m. for 10 minutes, the supernatant fluids were removed and passed through sand and pulp filters. The fluid that had passed through the sand and pulp filter was then filtered through graded collodion membranes under 2 atmospheres pressure of nitrogen. The filtrate was injected into the groin of guineapigs in doses of 1 c.c. All guineapigs were subsequently tested for immunity a month after the injection of the filtrate. The results are shown in Table III.

TABLE III.  
FILTRATION THROUGH GRADED COLLODION MEMBRANES.

Approximate pore diameter (A.P.D.).	Number of Tests.	Number showing Febrile Reaction.	Result of Immunity Test.
440	3	3	Immune
320	2	2	"
260	2	2	"
200	2	2	"
175	3	2	2 immune 1 not immune
141	2	1	1 immune 1 not immune
130	2	1	1 immune 1 not immune
114	2	0	Not immune
100	3	0	" "

Since the limiting membrane had an A.P.D. of 114  $m\mu$  the average size of the virus particles as determined by this method would lie between 38 and 57  $m\mu$ . DURAND failed to pass the virus through a membrane with an A.P.D. of 160  $m\mu$ ; this would give a size for the virus of 53 to 80  $m\mu$ .

#### CULTIVATION OF THE VIRUS.

The virus has been successfully cultivated in a medium consisting of one part of inactivated human serum and nine parts of Tyrode's solution containing two drops of minced chick embryo tissue. The original inoculum consisted of 0.5 c.c. of a 1 in 10,000 suspension of infected guineapig spleen in Tyrode's solution. This was added to 4.5 c.c. of serum-Tyrode's solution. Subcultures were made every 4 days, 1 c.c. being added to 4 c.c. of fresh medium



containing chick embryo tissue. Thirty consecutive passages were made; the last culture was found to be infective for guineapigs when 1 c.c. of a dilution  $10^{-6}$  was injected subcutaneously. After the fifteenth subculture there was a very gradual increase in the incubation period from 2 or 3 up to 4 or 5 days.

In order to determine how long virus could continue to survive in contact with the same chick embryo cells the following experiment was carried out. As before, 0.5 c.c. of a 1 in 10,000 suspension of infected guineapig spleen in Tyrode's solution was added to 4.5 c.c. of serum-Tyrode's solution and placed in the incubator at  $37^{\circ}$  C. Every week 1 c.c. of the supernatant fluid was removed and inoculated intraperitoneally into a guineapig, the incubation period, that is to say the time from injection till the development of a febrile reaction, being noted. The same amount of fluid as was removed every week was returned, no fresh cells being added. Virus was still present in the flask 77 days from the time the original culture was put up. The incubation period was gradually increased from 3 days to 14 days. Since chick embryo cells cease to respire after about 3 days when kept in serum-Tyrode it is obvious that virus D continues to survive, if not to multiply, for many days after the metabolism of the cells has ceased. Further studies are necessary to determine the titre of the virus during its period of survival. Virus D, placed in serum-Tyrode as before, but without any chick embryo cells, survived at  $37^{\circ}$  C. for 7 days but the incubation period was 14 days after the injection of 1 c.c. of the culture fluid. No virus was present after 14 days.

Virus has been inoculated on to the chorio-allantoic membrane of the developing chick embryo, aged from 9 to 10 days, and directly into the yolk-sac. Six passages were made into the yolk-sac, using yolk-sac ground up in physiological saline diluted 1 in 10. Both after injection into the yolk-sac and on to the chorio-allantoic membrane, death of the chick embryo occasionally took place 3 to 4 days after inoculation. Virus was present in the extra embryonic fluids and in the internal organs, of the developing chick embryo.

#### IMMUNITY.

In the guineapig after a febrile reaction immunity develops to a further inoculation of 1 c.c. of a 1 in 10 suspension of infected guineapig spleen. This immunity has been found to be present within three weeks of the end of the febrile period and to be present in guineapigs 6 and 12 months after inoculation. In one instance a female guineapig inoculated 12 months previously was immune, while her two offspring, injected 5 days after birth, were also found to be immune. These two baby guineapigs are the only guineapigs, among more than 200 injected not actually inoculated with virus D, that have been found to be immune.

In contradistinction to the solid immunity against reinoculation, the development of virucidal immune bodies in the serum is not as large as might be expected. In order to demonstrate virucidal immune bodies dilutions

of virus from 1 in 10 to 1 in 10,000,000 were mixed with equal amounts of serum from recovered guineapigs; control tubes contained the same amount of virus mixed with normal guineapig serum. The mixtures were placed for 1 hour in the incubator at 37° C. and then overnight in the ice-chest. They were injected into the groins of guineapigs in 1 c.c. amounts. Serum taken from recovered guineapigs 1 month after inoculation usually neutralized dilutions of 1 in 10,000, but not lower dilutions. It was noticeable, however, that in the dilutions that were just not neutralized there was usually a prolongation of the incubation period.

The serum of G.M.F., 2 months after an attack, neutralized virus in a dilution of 1 in 10,000; 16 months after infection with virus D the serum neutralized the virus in a dilution of 1 in 2,500,000 but not in a dilution of 1 in 500,000.

Two laboratory technicians who had handled virus D at no time showed any neutralizing power.

The serum of a cat inoculated intracerebrally was found 4 weeks later to neutralize virus in a dilution of 1 in 500,000 but not in 1 in 5,000; a second cat, however, failed to neutralize in a dilution of 1 in 500,000. The serum of a dog 6 weeks after intracerebral injection neutralized a dilution of virus 1 in 500,000 but not 1 in 5,000.

The serum of a rat 35 days after intraperitoneal inoculation failed to neutralize virus in a dilution of 1 in 500,000. A hyperimmune serum, capable of neutralizing virus in a dilution of 1 in 5,000, was prepared in the rabbit.

Successful complement fixation has been obtained using egg yolk-sac in saline.

#### *Chemotherapeutic Experiments.*

Sulphanilamide, sulphapyridine and sulphathiazole given in varying doses to infected guineapigs failed to delay or otherwise modify the reaction.

#### RELATIONSHIP OF VIRUS D TO OTHER VIRUSES.

In 1936 LAIGRET and R. DURAND, working in Tunis, described a virus isolated from mice and found in man in the course of immunization against yellow fever with mouse tissues. Unfortunately there is no precise account of the lesions produced by this virus in mice or in guineapigs. The latter animals, however, after an average incubation period of 6 weeks, had fever for about the same time, accompanied by loss of weight: about 40 per cent. died. Whether this virus was virus D or the lymphocytic choriomeningitis virus is unknown.

In order to determine whether there was any cross immunity between virus D and lymphocytic choriomeningitis virus, three guineapigs that had recovered from virus D were inoculated with lymphocytic choriomeningitis

virus, W.E. strain. They reacted and died from the lymphocytic choriomeningitis in the same way and in the same time as three guineapigs that were not immune to lymphocytic choriomeningitis.

Six mice were injected intracerebrally with 0.03 c.c. of a 1 in 10 suspension of virus D and 2 hours later with the same dose of lymphocytic choriomeningitis virus. These mice died in the same time as six normal mice inoculated with lymphocytic choriomeningitis virus alone. The failure of virus D to interfere with the pathogenic action of lymphocytic choriomeningitis virus was also demonstrated in guineapigs.

The virus of lymphogranuloma venereum, injected into the groin of guineapigs causes an intense haemorrhagic reaction with enlargement of the lymph nodes not unlike that due to virus D except that it comes on after a shorter period and lasts only for 3 or 4 days. Two guineapigs that had recovered after infection with virus D were injected into the right groin with 0.5 c.c. of a 10 per cent. suspension of mouse brain infected with the virus of lymphogranuloma venereum. Two normal guineapigs were similarly injected. All four guineapigs showed typical bubo formation. Twelve mice were inoculated intracerebrally on two occasions at an interval of 10 days with 0.03 c.c. of a 10 per cent. suspension in physiological saline of guineapig spleen infected with virus D. 10 days after the second injection the twelve mice were divided into three groups and injected intracerebrally with 0.03 c.c. of dilutions  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  of mouse brain infected with lymphogranuloma venereum. Control mice were similarly injected with the same dilutions of the virus. Both sets of mice behaved similarly, showing that no protection was afforded against lymphogranuloma venereum by previous injections of virus D.

Two virus infections of guineapigs, however, show some similarity to virus D. In 1938 TEN BROECK and NELSON noted that certain young animals in their guineapig colony were febrile. Inoculation of organ suspensions from these febrile animals into normals gave rise to a fatal disease. After an incubation period of 2 to 5 days the temperature rose and remained high for approximately 5 days; death invariably took place about 2 weeks after inoculation. Leucocyte counts showed from 3,000 to 5,000 cells while the red blood corpuscles were normal. At death the kneefold lymph nodes were enlarged and reddened, the stomach was collapsed and the abdominal cavity was too large for its contents. The abdominal and thoracic viscera showed no characteristic gross pathology. Pneumonia was absent except in animals inoculated intranasally or in those infected by contact. When guineapigs also carried haemolytic streptococci or *Bacillus bronchisepticus* an extensive pneumonia was present. The combined infection of the virus and the haemolytic streptococcus could be transmitted by contact.

A proportion only of young white mice inoculated by various routes died. Intracerebral injection into rabbits caused a fever of short duration; cutaneous inoculation produced only a fever of short duration; intratesticular injection

caused an orchitis in one rabbit and subinoculation into rabbits then invariably caused an orchitis. Intraperitoneal injection into two cats was negative. In the serum of guineapigs the agent was still present when 1 c.c. of a dilution  $10^{-5}$  was injected. Heating for 20 minutes at  $60^{\circ}$  C. caused inactivation. The agent was filterable through Berkefeld N and W filters and was cultured for eighteen passages on the chorio-allantoic membrane of the developing chick embryo.

More recently BEEUWKES (1940) in Batavia inoculated the blister contents from a case of dermatitis dolorosa polymorpha (Brocq-Duhring's disease) into guineapigs which reacted with fever. Further experiments showed, however, that the agent did not derive from the blister fluid but from the apparently normal guineapigs which were harbouring a latent virus. Once activated by animal passage it caused fever, haemorrhage into the peritoneal cavity and slight congestion of the lymph nodes. Only a few animals died. The virus was inactivated by heating at  $65^{\circ}$  C. for 15 minutes. The blood showed a slight leucopenia. The incubation period was from 7 to 11 days. The lungs, brain, liver and other organs showed no specific changes. If the characteristics of these three infections are compared with the symptoms in Durand's disease it will be seen that although there are many similarities there are also certain differences which suggest that the four viruses may be strains of the same agent. The most important difference in virus D is that it is pathogenic for man as well as for the guineapig. Whether virus D is widely distributed as a cause of sickness in man or whether the infection is limited to laboratory workers whose contact with guineapigs is apt to be intimate, requires further investigation. Against the view that virus D is a pathogen of guineapigs is the failure to find resistant animals among normal stock and the fact that it does not spread by contact.

#### CONCLUSIONS.

A new virus infection of man and animals is provisionally termed Durand's disease.

A case due to laboratory infection is described. The symptoms consisted of fever, headache, nasal catarrh, cough, nausea and vomiting of blood. The virus is highly infective for guineapigs which react with fever, enlargement of the lymph nodes and spleen and interstitial pneumonia.

Guineapigs can be infected by subcutaneous, intracutaneous and intraperitoneal inoculation; by application to the scarified or even the normal skin, by intracerebral and intratesticular injection; by intranasal instillation. Intratesticular inoculation produces an orchitis with enhancement of virulence. Numerous other animals can be infected but after intraperitoneal inoculation the disease is usually inapparent. Rhesus monkeys, cats and dogs develop symptoms after intracerebral injection.

The virus is from 38 to 57m $\mu$  in size, as determined by filtration through gradocol membranes.

The virus has been grown in serum Tyrode and chick embryo and in the developing chick embryo *in vivo*. No cross immunity with lymphogranuloma venereum or lymphocytic choriomeningitis has been found.

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## A NOTE ON TRYPANOSOMIASIS OF GAME FROM TSETSE AREAS AT SHINYANGA AND UKEREWE PENINSULA.

BY

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The following records have been obtained by examination of thick and thin blood smears of various wild animals shot during the course of game control experiments on the borders of bush infested with both *Glossina swynnertoni* and *G. pallidipes* in the Shinyanga area. A few antelopes were also shot in tsetse-bush (*G. swynnertoni*) on the Ukerewe peninsula; these are included in the general observations and in the tables which give details of each animal examined.

WENYON (1926) summarized the findings of various workers in this field up to 1926, and although a certain number of observations have been added by investigators since then, data are still very scant, and insufficient for statistical treatment.

One of the objects of this work is the collection of such evidence as was indicated by SWYNNERTON (1936) where in his Appendix 5, note 2, he wrote "Close deduction (from results of recorded blood examinations) is not justified yet from the results of many workers differently obtained, but a single large-scale investigation of the bloods of the different species, by a standardized method and under the same conditions, may yet assist towards producing a picture of the relative usefulness of the different species of animals to the tsetse."

### TECHNIQUE.

Immediately the animal had been shot, its jugular was opened and one thick and two thin blood smears were taken. These were allowed to dry in the shade, and then brought into the laboratory. Thick and thin smears were then stained with Giemsa, and the second thin smear with a saturated solution of eosin in methyl alcohol.

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\*The writer is indebted to Mr. H. E. HORNBY for suggesting this line of investigation and for continued interest and advice. The majority of the animals were shot by Game Scout SIMBA FULO in the course of his routine duties around Shinyanga, but the writer also has to thank Capt. V. A. C. FINDLAY, Dr. C. H. N. JACKSON, and Mr. G. S. BRENT, all of whom were good enough to take blood-slides of animals they shot; other animals were shot by the writer. The staining and preliminary examination of all the slides were carried out by Mr. JAMES ZULA CHALI, a laboratory assistant of the department.

The thick smear was examined for trypanosomes, and at least 500 fields were searched before the slide was considered to be negative; if this was positive, the thin smear was then examined in order to obtain a clearer view of the trypanosomes. The eosin-stained preparation was used to measure the erythrocytes.

The trypanosomes found have been classed under one or other of the three main tsetse-carried trypanosome groups: *T. congolense*, *T. vivax* and *T. brucei*. *T. rhodesiense* and *T. gambiense* have not been recorded in the Shinyanga area.

The results of examining 135 animals shot between June, 1939 and September, 1941, are summarized in the table below:—

TABLE I.

	Number examined.	Number positive.	<i>T. brucei</i> .	<i>T. congo- lense</i> .	<i>T. vivax</i> .	Erythrocyte measure- ments.
Impala ( <i>Aepyceros melampus</i> ) ...	45	9	—	9	—	2.8-3.4
Roan Antelope ( <i>Hippotragus equinus</i> ) ... ..	11	1	—	1	—	3.0-4.6
Eland ( <i>Taurotragus oryx</i> ) ...	4	1	—	1	—	4.6-4.8
Topi ( <i>Damaliscus korrigum</i> ) ...	3	0	—	—	—	4.3-4.6
Giraffe ( <i>Giraffa camelopardalis</i> )	21	10	?	9	3	4.7-5.5
Greater Kudu ( <i>Strepsiceros strepsiceros</i> ) ... ..	2	2	?	2	1	5.6
Hartebeest ( <i>Alcelaphus cokei</i> )	1	1	—	—	1	3.9
Zebra ( <i>Equus quagga</i> ) ... ..	4	1	—	1	—	5.0-5.7
Duiker ( <i>Sylvicapra grimmia</i> ) ...	5	1	—	1	—	5.1-5.3
Dik-Dik ( <i>Rhynchotragus kirki</i> )	7	1	—	1	—	5.0-5.4
Steinbuck ( <i>Raphicerus camp- estris</i> ) ... ..	1	0	—	—	—	5.4
Thomson's Gazelle ( <i>Gazella thomsoni</i> ) ... ..	3	0	—	—	—	4.4-4.6
Reedbuck ( <i>Redunca redunca</i> ) ...	1	0	—	—	—	4.8
Wart-hog ( <i>Phacochoerus aethio- picus</i> ) ... ..	19	3	1	2	—	6.0-6.4
Serval Cat ( <i>Leptailurus serval</i> )	2	0	—	—	—	6.8-7.3
Genet Cat ( <i>Genetta tigrina</i> ) ...	1	0	—	—	—	6.1
Leopard ( <i>panthera pardus</i> ) ...	1	0	—	—	—	5.4
Spotted Hyaena ( <i>Crocuta crocuta</i> ) ... ..	1	0	—	—	—	—
Jackal ( <i>Canis adustus</i> ) ... ..	1	0	—	—	—	—
Hunting Dog ( <i>Lycaon pictus</i> ) ...	1	0	—	—	—	7.6
Baboon ( <i>Papio</i> spp.) ... ..	1	0	—	—	—	7.2
Total ... ..	135	30	1 & 2?	27	5	
Percentage ... ..		22.2	0.7	20	3.7	

The three main species in the above table show the following percentages of infection: impala 20 per cent., giraffe 47.6 per cent., and wart-hog 15.8 per cent. There are too few observations from the other species to include them in the comparison.

The percentage of the animals showing infection appears to vary according to whether the animal is young (under 1 year) or adult, and according to the season when shot. Unfortunately the data are insufficient to show whether these differences have any significance.

These tables suggest that the young animals show a higher percentage of

TABLE II.

Comparison between the numbers infected of young and adult animals, omitting Carnivores and Primates.

	Adults.		Young.	
	Negative.	Positive.	Negative.	Positive.
Impala ... ..	32	6	4	3
Giraffe ... ..	10	10	1	0
Wart-hog ... ..	7	0	6	3
Others ... ..	33	7	1	1
Percentage . . .	21.9		36.8	

TABLE III.

Comparison between the numbers infected, bi-monthly through the year, in the Shinyana area.

	Jan.-Feb.		Mar.-Apr.		May-June		July-Aug.		Sep.-Oct.		Nov.-Dec.	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Impala ...	9	2	3	1	1	2	18	2	2	0	1	2
Giraffe ...	3	1	0	4	1	1	6	3	1	0	0	1
Wart-hog ...	3	0	1	0	5	0	5	1	1	1	1	1
Others ...	2	1	1	2	7	1	11	1	4	2	4	0
Percentage infected	19.0		58.3		22.2		14.9		27.3		40.0	

trypanosomes in the blood than the adults, and that the percentage infected is higher during the rains than the dry season. The short rains commence usually about the beginning of November and continue to the end of December,



when there is a short dry spell during January to February. (Some rain does fall in this period.) The so-called long rains start during March and have finished by the beginning of June. No rain falls during July to October.

It is highly probable that all wild animals, except monkeys, rodents and small insectivores become infected with trypanosomes while young (within the first six months). However, their natural resistance overcomes the infection, and although they may retain a few trypanosomes in their bodies these would be too few to be found in a blood slide. Heavy rain storms cause the animals to get wet and cold, which brings about a relapse. Both the kudu shot were obviously sick animals and showed very heavy mixed infections.

#### SUMMARY.

(1) Wild animals shot in tsetse areas of Shinyanga (*Glossina swynnertoni* and *G. pallidipes*) and Ukerewe peninsula (*G. swynnertoni*) have been examined for trypanosomes.

(2) The results of examining a number of individuals from twenty-two animals are given in Table I. A total of 22.2 per cent. of all the animals examined showed trypanosomes in their bloods; of these 0.7 per cent. showed *T. brucei*, 20 per cent. *T. congolense* and 3.7 per cent. *T. vivax*. A few animals showed mixed infections.

(3) Of forty-five impala examined 20 per cent. were infected; of twenty-one giraffe examined 47.6 per cent. showed infection, and of nineteen wart-hog 15.8 per cent. were infected.

(4) Tables II and III give figures that suggest that the young animals show a higher per cent. infection than the adults, and that a higher per cent. infection is found during the rains. These figures, however, are insufficient for statistical treatment, and therefore must be taken with reserve.

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## NUTRITIONAL GLOSSITIS AND VITAMIN B<sub>2</sub> THERAPY.

BY

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In the Gold Coast there prevails a type of superficial glossitis which appears to be caused specifically by lack of riboflavin in the diet. Gold Coast diets generally are rather poor in riboflavin-rich foods, milk, eggs and meat. This is especially true in boarded institutions, in schools and in prisons. The cases to be described have been encountered in the course of nutrition surveys conducted in the Gold Coast during 1940. Many cases of superficial glossitis have been seen, especially in the large boarding school centre, Cape Coast town. The complaint is well known in all the schools and is associated with change from the home diet. Itching of the scrotum is a common accompanying symptom.

Early in 1940 several pupils suffering from sore tongue were treated with 100 mg. nicotinic acid daily, but without benefit. While under treatment one boy's tongue and lips became acutely red and more painful than they had been before. This treatment was therefore stopped. The results of treatment of glossitis with marmite by LANDOR (1935) in Malaya suggested that riboflavin therapy might be efficacious in the Gold Coast cases. After long delay a supply of lactoflavin was obtained. The results were dramatically successful.

The ailments and course of treatment of six typical cases are set out below. The boys were drawn from three different schools and were treated in consultation with Dr. EDDY, Medical Officer of Health, Cape Coast.

### CASES.

*Case 1.* J. K. A. Suffering from an extensive patch of glossitis and slight scrotal dermatitis. Received 100 mg. nicotinic acid daily. On 4th day felt soreness in the throat on swallowing. Stomatitis developed; a zone of inflammation appeared between apposed surface of lower lip and gum. Nicotinic acid substituted by riboflavin 5 mg. daily. Glossitis and stomatitis cleared up completely in 6 days.

*Case 2.* C. O. Suffers from sore tongue every year. Tip of tongue denuded. Slight scrotal dermatitis. Received 100 mg. nicotinic acid daily. By 5th day suffered pains on swallowing. On inspection, tongue redder. Lactoflavin administered. Seven days later tongue and scrotum completely healed.

*Case 3.* J. A. Slight glossitis and scrotal dermatitis. Received 5 mg. riboflavin daily. Clear of symptoms in 1 week.

*Case 4.* A. A. Has suffered from sore tongue and itchy scrotum on and off for several years. Tongue normal now, but scrotum shiny and red, the rugae have disappeared. Received 100 mg. nicotinic acid daily. Scrotal symptoms rapidly became worse. On substituting riboflavin 5 mg. daily, the symptoms cleared up in a few days. (This pupil had been treating his complaint successfully with marmite during vacations.)

*Case 5.* J. X. A. Suffering from large denuded patch on tongue, of recent onset; this is his first year at school. Symptoms rapidly subsided on treatment with 5 mg. lactoflavin daily. All clear by 7th day.

*Case 6.* J. F. B. First term at school. Sore tongue and scrotal itch began about 3 weeks after entering school. Red patch on dorsum, filiform papillae disappeared. Slight scaling of scrotum. Received 5 mg. lactoflavin daily. Symptoms subsided by 4th day, completely clear by 13th day.

The cases above described were mild and typical. (More severe cases, with cheilitis, are to be seen in some of the prisons). The symptoms and signs are typically as follows :—

The pupil experiences pain in the tongue, especially on taking hot foods, particularly soup containing peppers. On examination the tongue is seen to be red as the loose epithelium has already been shed. Later, in advanced cases, the filiform papillae disappear and the tongue becomes smooth. In many cases numerous small fissures appear on the dorsum also. Scrotal symptoms begin about the same time as the glossitis and the skin begins to flake slightly; but this condition is seldom severe among the schoolboys. These symptoms wax and wane, subsiding during vacation and relapsing during term.

Having identified the missing factor, riboflavin, it would seem easy by study of the school diets to perceive the qualitative defects; but beyond an obvious lack of the riboflavin-rich foods, milk, eggs and fresh meat, little criticism can be made, as the riboflavin content of the popular local foods is unknown.

In order to compare home diets with the school diets many of the pupils suffering from glossitis were requested to describe carefully their diet while at home and to contrast it with that received at school. The school managers co-operated courteously, but the results were disappointingly vague. The clue hoped for was mention of a higher consumption of *fresh* meat or *fresh* fish while at home; but few stressed this difference (which we know in many cases holds true). Most boys stressed faulty preparation of the school meals, and the lack of fresh vegetables; yet, this possible association with glossitis is obscure as green vegetables are stated to contain very little riboflavin.\* Correction of the dietetic faults must therefore await further experimental therapy with natural foodstuffs.

#### DISCUSSION.

The symptoms caused by riboflavin deprivation alone are apparently variable, and depend on the severity of the deficiency. SYDENSTRICKER *et al.* (1940) found that cheilitis, tongue changes, seborrhoeic dermatitis and perikaritis developed rapidly in patients on diets deprived *completely* of riboflavin.

\* Recent work reveals that spinach is a rich source of riboflavin.

Other observers have not reported corneal inflammation, possibly because in practice one is dealing with *partial* deprivation. In Malaya, LANDOR (1935 and 1939) has described a syndrome most closely resembling that prevalent in the Gold Coast; superficial glossitis with scrotal eczema, and in some cases, dimness of vision, all responding to treatment with marmite. Later, LANDOR (1939) treated ten cases of scrotal eczema with 500 mg. nicotinic acid daily. A few cases showed improvement but four became much worse and four others developed sore mouths. Yet all were rapidly cured on exhibition of  $\frac{1}{2}$ -oz. marmite (which is rich in riboflavin) daily.

In Palestine, KATZENELLENBOGEN (1939) obtained good results in 21 out of 24 cases of glossitis with 100 mg. nicotinic acid daily. The other three cases did not improve.

In India, AYKROYD (1939) obtained good results in 16 out of 24 cases of glossitis. In the remaining 8 there was little change.

It is of interest that none of these workers in the tropics has obtained constantly good results with nicotinic acid in the treatment of endemic nutritional glossitis. A proportion of their cases failed to respond or became worse. The apparent conclusion is that mild chronic superficial glossitis may be caused by lack of either nicotinic acid or riboflavin (or doubtless by lack of both factors). Consequently it seems that in cases where one form of therapy fails the other should be tried. There appears to be a riboflavin deficiency syndrome and a nicotinic acid deficiency syndrome; and glossitis may be the sole minor symptom presenting in either condition. When glossitis occurs as part of the syndrome described by LANDOR, with cheilitis and scrotal eczema, and perhaps dimness of vision, the symptoms may be caused solely by lack of riboflavin (as they are all cured by marmite but not by nicotinic acid). When the glossitis occurs together with diarrhoea and pellagroid dermatitis, all responding to treatment with nicotinic acid, deficiency of this factor is the specific cause. Both deficiencies are variable components of pellagra. Accordingly, it may be practicable in future to diagnose pellagra glossitis or sprue glossitis in terms of the precise causative deficiency, by therapeutic tests.

The symptom, dimness of vision, calls for special mention. In Nigeria, FITZGERALD-MOORE (1935-1936) described cases of dimness of vision going on to post-optic atrophy, but responding well to treatment with marmite in the early stages. LANDOR's patients also suffered from a similar dimness of vision. In Sekondi, Gold Coast, 1937, my predecessor and I treated several patients, women advanced in pregnancy, suffering from dimness of vision of recent onset. These cases rapidly responded to treatment with marmite; but it was of interest that none of them presented any of the other symptoms of vitamin B deficiency.

It is remarkable that SYDENSTRICKER did not record impairment of vision in patients experimentally *completely* deprived of riboflavin. The explanation

may be that the interval between deprivation and resumption of riboflavin was too brief for visual symptoms to develop; nerve changes develop slowly.

#### SUMMARY.

The successful treatment of six cases of superficial glossitis with synthetic riboflavin is described. The aetiology of nutritional glossitis as ascertainable by therapeutic tests is discussed.

Recent analyses reveal that spinach is a rich source of riboflavin. It is therefore of interest that fresh greens are remarkably deficient in the school diets, although regularly provided in the home diet.

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## THE NAME OF THE NONPERIODIC *WUCHERERIA* OF THE PACIFIC.

BY

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Sir PHILIP MANSON-BAHR (1942) upholds his designation (1941) of the nonperiodic *Wuchereria* of part of the Pacific, to which Dr. G. CARMICHAEL Low (1941) had objected. His designation should not be accepted without question. He admits that he has overstepped the bounds of zoological propriety in proposing the new specific name of "*Wuchereria pacifica*," but in extenuation urges three points: the "almost inextricable medley of names with which tropical medicine has become encumbered mainly by the efforts of systematic zoologists," the gross infringement of the Code of Zoological Nomenclature that has taken place, and the statement that its application cannot always be upheld. So it becomes necessary to consider the Code—first rejecting the implication that one impropriety justifies others.

### I. THE CODE OF ZOOLOGICAL NOMENCLATURE.

The formation of the Code was due to the action of systematic zoologists at International Congresses. At the Third Congress in 1897 an International Commission on Zoological Nomenclature was set up, it reported to and was enlarged at the Fourth Congress in 1898, and drew up the Code which was adopted at the Fifth Congress in 1901. A six years' study before adopting the Code measures the seriousness with which the Commissioners viewed their task.

An essential feature of the Code is the Law of Priority, by which the first name given to an animal under the articles of the Code becomes its valid name. But the determination of priority may not always be easy, so CH. WARDELL STILES had in the *Zoologischer Anzeiger* of 15th January, 1910, an open letter in which these words occur: "A number of zoologists have expressed the opinion that a list of the most common zoological names should be prepared, and that the International Congress of Zoology should accept this list in the future as free from any operation of the Law of Priority." The setting up of this Official List was approved by the Graz Congress in 1910, and decisions on the List's contents have been given in a number of "Opinions." In Opinion 66 of 1916 there were added to the Official List the generic names *Ancylostoma*, *Ascaris*, *Dracunculus*, *Gnathostoma*, *Necator*, *Strongyloides* and *Trichostrongylus*, they having been accepted by the Commissioners with no dissentient vote.

As an example of the almost inextricable medley of zoological names

with which he holds tropical medicine to be encumbered, MANSON-BAHR (1941) writes, "I need hardly mention the case of *Bilharzia*, *Schistosoma* and *Schistosomum*, for it is not yet settled to which sex this sexually-minded trematode belongs." For systematic zoologists this fluke's name was settled 20 years ago; the history has its lessons. Before 30th September, 1858, WEINLAND gave to the fluke the Greek neuter name *Schistosoma*, DIESING followed later in the same year with *Gynaecophorus*, COBBOLD in 1859 with *Bilharzia*, MOQUINTANDON in 1860 with *Thecosoma*, R. BLANCHARD in 1895 changed the Greek neuter into the Latin neuter *Schistosomum*, while BRADY in 1877 complicated matters by giving the name *Schistosoma* to an arachnid. Here is a good example of the chaos which comes about when each author rejects zoological names according to his own personal wishes (STILES, 1905). The Commission's work was very different; before placing this name on the Official List it had consulted by post 350 zoologists and zoological institutions, and in no answer from them was any objection made to *Schistosoma*, so the Commissioners adopted that name with no dissentient vote among themselves, and it became the valid name under the Code as reported in Opinion 77 (1922). The position then is that some 80 years ago the generic name of the blood fluke was a chaos, that systematists made order of it 20 years ago, in this case by insistence on the application of the Law of Priority, that all medical men have not accepted, or appreciated the welcome ruling, but that some are still blaming systematists for an ancient chaos put straight by them 20 years ago.

On another point there seems to be confusion. There is no reason for the name of a disease to change with that of the parasite that causes it. Medical and zoological nomenclatures are fully independent. It is appropriate to commemorate in the name of the disease the short 37 years of the brilliant life of BILHARZ while still insisting that the parasite is *Schistosoma*; when speaking of threadworm or pinworm infection one may yet bear in mind that the parasite was named *Enterobius* by LEECH (in BAIRD, 1853), nor need trichinosis be otherwise named because, though OWEN gave the parasite the name *Trichina* in 1835, MEIGEN had already given this name to a dipteran in 1830, so that in 1895 RAILLIET had to change it to *Trichinella* since no two animal genera may have the same name.

Accordingly this position is reached. Zoological nomenclature is as strictly a matter for zoologists as medical nomenclature is for medical men, a point brought out by STILES (1900) in a paper entitled "The use and abuse of zoological names by physicians." The "almost inextricable medley of names" with which tropical medicine has been encumbered mainly by systematists dates from a time before that at which the Code of Zoological Nomenclature was finally approved by systematists in 1901. By systematists medley has in fact been made to give place to order. Basing themselves on the Code they have published their findings as "Opinions," and the particular instance of muddle mentioned by MANSON-BAHR became order for systematists 20 years ago, and should before now have become so for medical men.

The statements that in matters affecting tropical medicine there has been infringement of the Code since it came into being in 1901, and that the Code cannot always be upheld are unsupported generalities which of necessity cannot be met; nor should the implication, that one infringement justifies a second, appeal to those who wish to keep order achieved from slipping back into chaos.

In zoological classification another point should be in mind. *Homo sapiens* ought to be the standard determining the limits within which there may be variations among the members of one zoological species. Man differs from man in shape, colour and habits, yet not even the *Herrenvolk* have, so far as I know, decided that we others are not co-specific with them, but grade us as merely lesser breeds of *Homo sapiens*.

## II. THE STANDING OF "*Wuchereria pacifica*" IN NOMENCLATURE.

This proposed species is not based on morphology, for its microfilaria has not been admitted to differ from that of *W. bancrofti* in other parts of the world. Of its adult forms from Fiji, Professor LEIPER (in BAHR, 1912b, p. 36) reported that they belonged to the species *Filaria bancrofti* Cobbold, 1877; regarding those from Polynesia, BUXTON (1928, p. 71), who sent them to him, reported that LEIPER was not at present prepared to give an opinion on their systematic position. I have traced no further opinion by LEIPER, nor does MANSON-BAHR record one now. Instead, he discards distinctive morphology and bases his species on these features—(1) nonperiodicity of larvae in skin blood; (2) "certain distinctive clinical features" (MANSON-BAHR, 1941), or "certain distinctive biological peculiarities" (MANSON-BAHR, 1942); (3) a defined geographical distribution; (4) a distinctive intermediary host.

### 1. PERIODICITY OR NONPERIODICITY.

If the biological or physiological activity which controls the times at which the filarial young are found in the skin blood were accepted as a good basis for species differentiation, there would be no logical justification for limiting this new basis to that which escapes from the genital passage. That which is expelled from the rectum must be given equal weight. The acceptance by all prisoners in an Indian jail of the stimulus of the call to the early morning parade, their periodic morning flocking into the latrines and their periodic filling of the latrine pans would, on these lines justify the conclusion that man had thereby not merely classified but had created a new species of *Homo*. I cannot accept it.

### 2. "CERTAIN DISTINCTIVE CLINICAL FEATURES" OR "CERTAIN BIOLOGICAL PECULIARITIES."

In the later and amended wording this matter is given a biological, in place of its earlier clinical, status. What is actually being stressed is a difference in the usual siting of certain physical signs when the periodic and non-periodic infections are compared. In the non-periodic form elephantiasis of arms and



breasts and enlargement of epitrochlear lymph nodes are commoner, while lymphuria and chyluria are rarer than in the periodic form. These local physical signs I believe\* to be reactions of the host to the infective larva which enters him from the mosquito, to the adult into which it grows and to the young it produces if a fertilized female.

I have pointed out (LANE, 1932) that after an infective larva from a mosquito pierces man's skin it may be carried away on the blood, or on the lymph, escalator. If on the former it may reach any part of the body, and any symptoms or signs it may cause directly or indirectly will be no pointer to the place of invasion. Should, however, it board the lymph escalator, the adult into which it grows is found mainly in a lymph node or in its branching lymph afferents, so that any effects it may produce in areas which drain into those nodes are pointers to the sites where infective larvae originally entered the skin.

By this reasoning the infective larvae which carry a non-periodic infection have been brought by mosquitoes mainly to those areas of the skin which drain into axillary lymph nodes, those which carry a periodic infection mainly into those that drain into retro-abdominal nodes. This might happen either because the usual respective insect hosts have a preference for feeding on different parts of the body (of that I think there is no evidence), or because the habits of man in different localities expose different parts of skin to the mosquito. Regarding this latter, BUXTON writes that in the old days dress was similar for men and women and that nothing was worn above the waist; but that now Samoans generally dress in imported fabrics, and Western conventions as to the exposure of women are observed, but that these conventions are hardly a part of native life though held to in the presence of a foreigner.

In the old days there was then a habit among Samoans which fully exposed to day-feeding mosquitoes the skin above the waist, and the inference is that when among themselves this habit still holds. Of course this inference needs confirmation, but subject to this it explains the greater incidence of evidences for lymph obstruction in skin areas draining into the axillary lymph nodes in a locality which happens to be one of non-periodic infection, and it does so on habits that merely influence relationship between host and mosquito.

In other words the "distinctive biological peculiarities" of "*Wuchereria pacifica*" depend on habits of man and mosquito, are not attributes of the worm itself, and accordingly may not be used as grounds for making even a biologically based species of the non-periodic form of *W. bancrofti*.

### 3. A DEFINED GEOGRAPHICAL POSITION.

BUXTON (1928) put the part of the Pacific in which non-periodic infection prevails as lying east of Longitude 170° E. WOODMAN and BOKHARI have now reported (1942) that, of twelve infections by *W. bancrofti* met with in the Sudan a quarter were non-periodic. On this evidence this specific character does not hold.

\* Evidence now being arranged for publication.

## 4. A DISTINCTIVE INTERMEDIARY HOST.

In the matter of filariasis in Polynesia, BUXTON (1928) wrote, "This absence of periodicity was an anomaly which continued to exercise the mind of Sir PATRICK MANSON for many years; it was largely for this reason that he and the London School of Tropical Medicine sent no fewer than three expeditions to Fiji and Polynesia." The wording seems to imply that the periodic is the prior and primitive form, and with this falls in the suggestion by BUXTON that there was little doubt that the Polynesians originated in Malaya and that the form there is periodic and so prior. He adds, however, that, until the Europeans came, there was frequent coming and going between islands and archipelagoes. That being so, disease might have spread in either direction.

A number of considerations seem to point to the priority of the non-periodic form. If that were the sequence, a simple habit of life with microfilariae in the blood at all hours evolved into a more complex one in which they are in it at certain hours only in arresting numbers, and those are the hours at which a certain common intermediary host feeds freely. There are in Polynesia several races of *Aedes variegatus* all easy of infection, a character which seems essential for the first establishment of parasitic life; indeed so easy of infection is this species that individuals are apt to die under its weight. But it is the case that in suitable conditions the periodic form infects *Aedes* as heavily as it does the non-periodic.

"I was able to study the development of non-Fijian *Microfilaria bancrofti* in *Stegomyia pseudoscutellaris* on three separate occasions and to determine the efficiency of this insect as regards this variety of the filaria. The mosquitoes were induced to feed at night by placing the cages in a strong light after a period of starvation. All stages of development were exactly similar to those observed in the Fijian variety and the ingested filariae developed, both as regards numbers and rapidity in the same remarkable way." (BAHR, 1912a, p. 141.)

From the measures needed to entice the insects to feed, it follows that by the term "non-Fijian" was meant periodic. Under the Law of Priority *Stegomyia* has given place to *Aedes* as generic name and *pseudoscutellaris* has become a race or subspecies of *variegatus*. That the intermediary host is "distinctive" is merely another way of saying that certain habits fit together, the feeding habit of *A. variegatus* and the habit that produces a fairly uniform hourly content of microfilariae in the skin blood. On the other hand it was shown 30 years ago that the feeding habits of this mosquito can be made to alter so that they fall in with the hours at which the microfilariae swarm in the skin blood in the periodic form of infection, and that when this has been brought about, development in this mosquito is equally good whether the microfilariae were of the periodic or non-periodic form. As between the two forms the power to infect *A. variegatus* is the same. It is not distinctive for one or the other.

On analysis, then this basis for the proposed new species is that its young

are present in the food of this mosquito at all instead of only at certain hours ; in other words it concerns the difference between periodicity and non-periodicity—and that point has already been considered.

### SUMMARY.

In general medical men have no just cause for complaint against zoologists for changes made in the names of parasitic worms ; but some medical men have failed to make themselves acquainted with, or to take advantage of, the release (in this matter of zoological names) from the Law of Priority which is part of the International Code of Zoological Nomenclature. The name *Schistosoma* has been cited as an instance of the medley produced by systematic zoologists ; in fact, it is an instance of release from the Law of Priority brought into effect 20 years ago after the International Commission on Zoological Nomenclature had put the matter before some 350 zoologists and zoological institutions before placing it on the Official List of exemptions.

In contrast the zoological species "*pacifica*," whose morphology does not permit of its being distinguished from *bancrofti*, is proposed by a single person on four abnormal grounds and is deliberately set outside the Code. These four grounds are, periodicity or its absence, certain distinctive clinical features, a defined geographical position, and a distinctive intermediary host. These have been considered in detail with the conclusion that they are not there or are not valid. So *Wuchereria bancrofti* remains the valid name.

Let us not start another era of nomenclatural chaos by making the naming of any parasite of man subjective and personal instead of objective and international.

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## CORRESPONDENCE.

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### ZOOLOGICAL NOMENCLATURE AND MEDICAL SCIENCE

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*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

I have read with interest the communications on this subject by Dr. G. CARMICHAEL LOW (1941) and Sir PHILIP MANSON-BAHR (1942), and though not wishing to take part in the discussion in any controversial spirit, perhaps I may be allowed, as a systematic zoologist, to mention one or two points.

The primary objects of the International Rules of Zoological Nomenclature were to promote stability and to avoid confusion in the names of animals. These ends are not easy of attainment, for all systematists will agree that there are often difficulties in the interpretation and application even of the rule of priority, but the establishment of separate sets of rules for specialized branches of zoology such as "medical zoology" and "parasitology," as suggested by Sir PHILIP MANSON-BAHR, does not appear likely to further them.

It does not seem to be explicitly stated in the Rules that species must be based on morphological characters. "Physiological species," "biological races" and the like are not generally recognized among the higher animals, but recently there has been a tendency to recognize such units among parasitic forms. Two main reasons for this are apparent: (1) the probability that the evolution of species in these groups proceeds, through the formation of "host-races," by adaptation to new hosts; (2) the fact that differences in host, habits and life-history are often more easily observed than very slight differences in morphology. The anatomical differences between closely-related forms may be very minute, and there are cases where expert morphologists have as yet found none, although physiological differences seem to exist. These cases present the systematist with a very difficult problem. The "type" system, on which all our nomenclatural rules are at present based, must break down if characters other than morphological ones are to be recognized as of systematic value.

Personally, I feel that "species" should not be recognized nomenclaturally unless and until they can be separated morphologically. But it would be somewhat arbitrary to deny the validity of a supposed "physiological" species until the morphological test had been very thoroughly applied. In such a case as Sir PHILIP MANSON-BAHR's Pacific filaria this would be no easy matter, for several reasons. It would involve the careful comparative study of a large amount of material, both adult and larval, of the forms from both areas of distribution by a competent specialist on the Nematoda. Small, random samples are not enough. An adequate amount of adult material would be difficult to obtain, and, as in other Filariidae, much individual variation would probably be found to exist. As Sir PHILIP said in his original article (1941), "the probability is that it [*i.e.*, '*Wuchereria pacifica*'] is a species in the process of evolution." This being so, it may not yet have developed any distinctive morphological characters, and I should prefer, with Dr. CARMICHAEL LOW, to regard it as a "race," a "variety," or at most a "subspecies" of *W. bancrofti* rather than as a distinct species, until the existence of a morphological *differentia* has been established. This would not mean that it could not be given a distinguishing name, but this should be trinomial, and, as Dr. CARMICHAEL LOW points out, might prove to be a synonym of a name already in existence.

Sir PHILIP MANSON-BAHR is rather disparaging of "the efforts of systematic zoologists," but I have no wish to cross swords with him, for I feel sure he knows that our unfortunately frequent changes in nomenclature are not nowadays merely capricious, and that there is no machinery for making the reasons for them understood by medical students and practitioners. "The case of *Bilharzia*, *Schistosoma* or *Schistosomum*" is not a question of "sex," and this particular Gordian knot has been cut by the International Commission on Zoological Nomenclature, who (*Opinion* 77, January 31st, 1922) placed the name *Schistosoma* on the "official list," rendering further discussion superfluous. Sir PHILIP draws one of his arguments from bacteriology, but I hope he does not hold zoologists responsible for that science or its nomenclature.

I am, etc.,

H. A. BAYLIS.

Department of Zoology,  
British Museum (Natural History),  
South Kensington.

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## LARVA MIGRANS.

*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

Dr. H. H. BAYLEY and Dr. G. H. WILDISH have written on the treatment of larva migrans. I have not had an opportunity of trying their methods, but in babies and children in whom it is difficult to keep on any sort of dressing for long and to keep it unsoiled, I have found alcohol injections useful.

A small bleb of absolute (or near absolute) alcohol is injected with a fine needle into or as close as possible to the advancing head of the larva. The first injection is generally successful in killing and thus immobilising the parasite. The alcohol often produces a small ulcer which heals readily.

If the injection registers a near miss the larva shoots off in the opposite direction, with the indignation of a rigid teetotaler.

I am, etc.,

CICELY D. WILLIAMS, D.M.

Trengganu,

Malaya.

6th December, 1941.

## REFERENCES.

BAYLEY, H. H. (1941). Treatment of larva migrans. *Trans. R. Soc. trop. Med. Hyg.*, **34**, 399.

WILDISH, G. H. (1941). Larva migrans. *Ibid.*, **35**, 129.



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